MYCOLOGIA

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NEIL EVERETT STEVENS, 1887–1949

C. L. SHEAR

Neil Everett Stevens was born April 6, 1887, in Portland, Me., and died in Urbana, Ill., on June 26, 1949. His ancestors were of English extraction. His father used to refer to the family as "Maineacs." His first ten years were spent at Portland where he attended the public schools. Unfortunately I have no record of this period, which must have been of great importance in determining his character and intellectual development. His early life being confined to the city, he missed the intimate contact with nature and agricultural operations without which the investigator of practical agricultural and horticultural problems is somewhat handicapped.

In 1897 the family moved to Auburn, Maine, where Neil graduated from high school in 1904. His education was continued at Bates College, where he graduated with the degree of A.B. in 1908. He then pursued graduate studies at Yale, receiving the Ph.D. degree in 1911. That he must have been above the average in his class is indicated by his election to Phi Beta Kappa and Sigma Xi. He took his collegiate honors lightly. He had no P.B.K. key until recently, when his family made him a present of one.

In 1914 he married Maude Bradford, also from Maine, who was his faithful helpmeet and companion to the end. There are three children, twin sons, Russell Bradford (Ph.D. Wisconsin), associate professor of botany, University of Tennessee; Carl Mantle

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2nd (Ph.D. Illinois), associate professor of chemistry in the State College of Washington, and Mary Christine, the wife of Lt. James McCray, U. S. Army.

He was a member of the following organizations: American Phytopathological Society, vice-president 1933, president 1934; Botanical Society of Washington, secretary 1928, president 1931; American Association for the Advancement of Science Council 1933; Mycological Society of America Council 1932; University Club, Urbana; Botanical Society of America, president 1948.

Neil, as his friends and coworkers familiarly addressed him, was my closest friend and associate in pathological and mycological investigation in the Bureau of Plant Industry for 24 years (1912–36). After so long and intimate an association one could hardly be expected to be satisfied with a mere formal record of the principal events of his life and work. Hence I hope to be pardoned if I indulge in some reminiscences of a more personal nature. Cut down so suddenly while very active and productive, his death came as a great shock to all who knew him. While attending the meeting of the Botanical Society of America at Chicago in 1948 as retiring president, he was stricken with heart disease but after some time in the hospital he was able again to undertake some of his University work. He retained much of his usual zest and cheerfulness. A quotation from his last letter to me in March 1949 is characteristic. He said: "Your letter of Feb. 3 was what I needed. I had been thinking for the last three weeks! I bet C.L.S. will want me to go up to Katahdin with him this summer and I don't feel like it. Now, however, that you don't feel like it either, that makes it all right. About 6 A.M., January 3, I did think the old gentleman with the scythe had come for me. However, I decided that I had already had twice as much fun in this life as any one was entitled to, which took some of the sting out. However, he decided otherwise and I have spent a month in bed. Honestly one of the happiest months you can imagine."

This indicates his general attitude toward life.

"After reading and discussing the various attempts of scientists and philosophers to solve some of the eternal mysteries of man and the universe, we came to the conclusion that the problem is beyond

the capacity and comprehension of the human mind in its present stage of development and that we must be content to contribute a few additions to our present fund of human knowledge and make such practical applications as are possible for the improvement of our physical and social condition, with faith in the belief that the practice of the golden rule will finally bring peace and happiness to the human race."

In one of his latest articles (Amer. Scientist **37**: 119. 1949) he explains his attitude toward scientific investigation and his method of approach, closing with approval the words of R. L. Stevenson: "To travel hopefully is a better thing than to arrive, and the true success is to labor."

The breadth and diversity of Neil's intellectual interests are well illustrated in a series of papers collected and published by Dr. Verdoorn in Chronica Botanica 1947 under the title, *Factors in Botanical Publication and Other Essays*, and in the rather complete bibliography appended.

As to religion, he was a member of the Methodist Church. His practical application of religion in daily life was humanitarian rather than creedal. His liberalism in theological matters is well expressed in an article on Dean Inge's views on science and religion (Science **63**: 281. 1926) in which he quotes with evident approval the following statement of the Dean: "I believe that in Science has come the chief revelation of the will and purposes of God that has been made to our generation."

He was very fond of outdoor life and exercise and as a member of the Appalachian Trail Club spent many of his holidays in breaking trails.

Though much interested in mycology and its history, his principal work was in plant pathology and especially cranberry diseases and related problems. His numerous publications in this field were of much scientific value and of great practical application. This work was greatly appreciated by cranberry growers everywhere. A very appreciative sketch of Dr. Stevens was published in "Cranberries" for December 1941 by the editor C. J. Hall.

He followed Dr. C. E. Bessey's advice to his botanical students: "Keep your mind in meristem condition." He was alert and ready

to absorb new knowledge and grow. He always approached a problem with an open mind and after reviewing all that had been published on the subject began observations and experiments to test previous conclusions and theories and to evolve new ones. He was no blind worshiper of authority. His studies in the history of science had shown how frequently the supposedly well established theories and conclusions of one generation may be replaced by the next and that absolute final conclusions can never be reached by mortal man; but that the joy of seeking and the pleasure of discovering something apparently new or useful was his greatest reward.

His first contribution to botanical literature was *Discoid Gemmae in the Leafy Hepatics of New England*, published in 1910 while he was doing graduate work under Dr. Evans of Yale. His next was in 1911 on *Dioecism in the Trailing Arbutus*. His Doctor's thesis was *Observations on Heterostylous Plants* published in 1912.

Under the stimulating influence of Dr. Wieland he became much interested in paleobotany, which resulted in a paper on *A Palm from the Upper Cretaceous of New Jersey*. His only mycological and pathological work at Yale was a course in forest pathology under Dr. Graves and his first paper in this field was on *Wood Rots of the Hardy Catalpa*. His broad training in different branches of botany and laboratory technique provided an excellent foundation for later specialization in mycology and pathology and a continued interest in all phases of plant science, both pure and applied.

Soon after graduation at Yale he received an appointment in the Botanical Department of Kansas State College where he taught one year. This experience broadened his views of the country and its agricultural problems. During this period he took a civil service examination for a position in Forest Pathology and to his surprise received an appointment in the Bureau of Plant Industry and was assigned to work on the chestnut blight project. It was here we first met and began working together in an attempt to discover the origin and relationship of the causal organism, *Endothia parasitica*. There was a difference of opinion among the investigators as to whether it was a variety or mutation of one of our native species

or a foreign species recently introduced. Our work together on this project as well as all the later work on which we published joint papers was so intimate that the contributions of each cannot be satisfactorily segregated. In general he made most of the cultures and cross inoculation experiments but also considered critically the taxonomic questions relating to the generic and specific concepts involved. We agreed that for practical taxonomic purposes species should be based on morphological, life cycle and cultural differences, which were proved by the comparison of an abundance of specimens from different localities.

It was soon demonstrated by means of specimens obtained from China by Mr. Frank Meyer, the agricultural explorer, and by those from Japan that the blight fungus was common on oriental chestnuts, but not a serious parasite, and that the fungus in this country was undoubtedly brought here on nursery stock. Here it found our native chestnut an easy victim and proceeded to exterminate it throughout the country. The result of this work was a monograph in 1917 of the species of *Endothia* thus far known.

In 1915 he was transferred to the office of fruit disease investigations and began work on strawberry diseases on which he published several papers, especially on the fruit rots.

Two years later he began his studies on cranberry diseases which were carried on in cooperation with the writer and other members of the office. This work he continued with slight interruptions for the remainder of his life, though restricted during his teaching career to his summer vacations. In carrying on the work on cranberries we visited all the cranberry growing districts in the United States and collected fungus and diseased material throughout the natural range of the two species, *Vaccinium macrocarpum* and *V. oxycoccus*, and at the same time all the fungi found on Ericaceous hosts, to determine their distribution and relation to those found on the cranberry. This work led us from the swamps of the Carolina coast to the summits of Mt. Washington and Katahdin, through the Adirondacks, Michigan and the Pacific coast of Washington and Oregon. At the same time all the available types and authentic specimens of the fungi reported on *Vaccinium* and closely related genera were studied. This led to the

herbaria of Peck, Ellis, Schweinitz, Curtis, Ravenel, Peters, and others. It also naturally led to an interest in the authors themselves and their methods of work, as such knowledge is essential in order to evaluate their results and publications. The search for biographical information led to visits to the homes and collecting grounds of the mycologists mentioned and to the accumulation of much information and many specimens. This work resulted in the publication of several biographical sketches (**12–16, 29, 32**) and a deep and permanent interest in the history of mycology in general and especially in America. A large collection of notes and letters have been accumulated from which we had hoped to prepare a history of mycology in America. In one of his last letters in reply to one in which I had urged him to retire and take up the history we had so long planned, he replied "We may get to that history of mycology yet."

In 1924 he began studies of *Botryosphaeria, Physalospora* and their pycnidial forms, *Dothiorella, Sphaeropsis,* and *Diplodia,* with a study of the currant blight and its cause *Botryosphaeria ribis chromogena.* The work on this fungus led to a thorough study of other species of *Botryosphaeria* and the closely related genus *Physalospora* and their life cycles and host relations. This involved cross inoculations and the examination of type material of the numerous species of *Sphaeropsis* and *Diplodia* which have been described. These species have been based largely on the host plant upon which they happened to be found. It was found that *Botryosphaeria* and *Physalospora* differ chiefly in their pycnidial stages, the former having *Dothiorella* and the latter *Sphaeropsis* or *Diplodia.* The results of this work were published in a series of papers (**19–27, 30, 34, 35, 38–40**). The winter of 1927–28 was spent in the Hawaiian Islands collecting fungi and studying fruit diseases. We made extensive collections on Oahu, Maui, Hawaii and Kauai and brought back more than a thousand specimens, mostly Pyrenomycetes. Only a small part of these have yet been studied although a joint paper (**27**) on the species of *Botryosphaeria* and *Physalospora* found was published.

In 1930 he went to England as a delegate to the International Botanical Congress at Cambridge, and studied strawberry and other fruit diseases and fungi in England, Scotland and Wales.

Some of the results of this work were published in a paper (**35**) on two Physalosporas found in England, thus clearing up the confusion in the life cycles of *Sphaeropsis malorum* Peck and of *S. malorum* Berk.

From 1930–33 he was in charge of the Plant Disease Survey in which he did much excellent work in improving methods of estimating crop losses from diseases and forecasting disease occurrence.

During 1934–35 he worked in the office of Cereal Crops and Diseases studying bacterial wilt of sweet corn. From 1931–36 he also acted as adjunct professor of botany at George Washington University. In 1935 he was a delegate to the International Botanical Congress in Holland. In 1936 he was offered the position of professor of botany at the University of Illinois. He considered and discussed for some time the question whether he could contribute more to the advancement of science and humanity in the Department or in the University. He finally decided in favor of the University, as it would not only give an opportunity to continue some of his research but also to put into practice some of his ideas for the improvement of college teaching. In spite of his rather youthful appearance and lack of the dignity supposed to characterize the conventional professor he was very popular and his informal manner and enthusiasm stimulated his students.

He was at work at the time of his death with his son Russell on a book entitled *Plant Pathology in Agriculture* which will be completed and published later.

In the premature passing of Dr. Stevens the family has lost a devoted husband and father; his students an inspiring teacher; his friends and acquaintances an enjoyable companion and science and education an able and productive contributor to their advancement.

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22. Botryosphaeria and Physalospora in the Eastern United States. Mycologia **17**: 98–107, illus. 1925. (With C. L. Shear and Marguerite S. Wilcox.)
23. The citrus stem-end rot "Diplodia"; its life history and relation to *Sphaeropsis malorum*. Phytopathology **15**: 332–340, illus. 1925.
24. The life history and relationships of *Diplodia gossypina*. Mycologia **17**: 191–201, illus. 1925.
25. Two species of Physalospora on citrus and other hosts. Mycologia **18**: 206–217, illus. 1926.

26. Occurrence of the currant cane blight fungus on numerous hosts in the Southern States. Mycologia **18**: 278–282, illus. 1926.
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THE NEED FOR THE PROBABLE ERROR CONCEPT IN MYCOLOGY *

NEIL E. STEVENS

Two major attempts to recognize the human element in science are well established. It is here proposed that, at least in mycology, the two be combined. First in point of time came the practice of adding to each specific binomial the name of its author. This was a frank, perhaps partly unconscious, recognition of the fact that no two authors would necessarily agree in delimiting a species and thus the binomial could be made meaningful only if its author was always named.

Stripped of the mathematical symbols which render it to some sacred and to others diabolical the probable error concept has the same human, almost humanitarian, basis. This is emphasized by the fact that Bessel, an early great student of probable error, preferred to call it "the personal equation" (**1**). Here too is a frank recognition of the fact that it is impossible to attain absolute accuracy. The probable error is also an attempt to indicate how reliable the measurements were, that is, how much we may depend on them. Like most statistical analyses it is another attempt to indicate the results which represent the true value which would be obtained if it were possible to make observations on all the individuals or cases. How badly just these aids—concepts—are needed in mycology is readily appreciated by those who have worked with long known, much named species.

The suggestion here made is merely that following the name of a new species there be added—perhaps by the editor of the journal publishing the description—a figure indicating the probability of its being indeed a good species. The exact method to be used

* This note was received for publication a short time before Dr. Stevens' death. The editor does not approve of being placed in the position of having to "rate" the new species proposed by other mycologists, but does recognize merit in the concept as applied to taxonomy. A. H. Smith.

can well be left to the mycological section of the next International Botanical Congress. Certain considerations are, however, obviously important. For example, the number of specimens examined in preparing the description, *i.e.*, their relation to the number probably existing in the world. Other considerations would be the known difficulty of the fungus group and for the older workers the previous record of the author. A graduate student studying his first fungus and deciding that it must be a new species probably should receive a charitably high rating; one, for example, that would indicate there was one chance in a hundred of his having a genuinely new species.

Taxonomy being as Hall and Clements (2) some years ago pointed out, ". . . the only field of science in which the blunders and banalities of the indifferent and incompetent are respected and perpetuated," much interest would be aroused over the "error indices" of various early workers. To Farlow, for example, who spent a lifetime of application and described some half dozen species, one could almost automatically assign an index indicating very low error.

To Berkeley who in a life devoted to the duties of an English parish, the education of thirteen children of his own, plus the conduct of a boarding school of thirty to thirty-five pupils and to mycology, and who described over five thousand new species the rating would of necessity be somewhat different.

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CHEMICAL AGENTS FOR THE CONTROL OF MOLDS ON MEATS[1]

FREDERICK T. WOLF AND FREDERICK A. WOLF

INTRODUCTION

The large amounts of spoilage of meat and meat products by molds during World War II made desirable consideration of the various fungi responsible and attempts to devise means of prevention. Species isolated from meats by previous workers (Bidault, **1921**; Brooks and Hansford, **1923**; Wright, **1923**, **1925**; Yesair, **1929**; Haines and Smith, **1933**; Semeniuk and Ball, **1938**; Empey and Scott, **1939**; Jensen, **1942**) include the following: *Alternaria tenuis, Aspergillus chevalieri, A. clavatus, A. niger, A. repens, A. ruber, Botrytis elegans, B. pellicula, B. rosea, Cladosporium herbarum, Dematium pullulans, Hormodendrum cladosporioides, Monilia sitophila, Monascus purpureus, Mucor lusitanicus, M. mucedo, M. racemosus, Oospora lactis, Penicillium anomalum, P. chrysogenum, P. crustaceum, P. expansum, P. melinii, P. notatum, P. puberulum* and various unidentified species of the asymmetrica-velutina group, *Rhizopus nigricans, Sporotrichum carnis, Thamnidium chaetocladioides* and *T. elegans*. It is evident from these lists that those molds usually present in the air and soil are most commonly responsible for meat decay.

It has long been recognized that temperature and humidity are primary factors in relation to the keeping of meats; hence most efforts at meat preservation have involved such purely physical methods as refrigeration and drying. The present study, however, is restricted to a consideration of chemical preservatives, and the inhibition of mold growth through chemical means. Regulations by the Bureau of Animal Industry limit chemical preservatives of

[1] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number *274* in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

meats sold for human consumption in the United States to the following agents: Common salt, sugar, wood smoke, vinegar, spices, saltpeter, $NaNO_3$, $NaNO_2$ and sodium benzoate.

Any study of chemicals for prevention and control of mold on meats should include firstly basic laboratory studies using a representative sampling of pure cultures of fungi to determine concentrations of the various chemical agents which are inhibitory to the various organisms. Such studies should be followed by toxicological and pharmacological tests, to determine whether the chosen chemicals are safe for human consumption. Finally, technological studies, involving means of application of the chosen chemicals, and large scale tests under diverse environmental conditions of the keeping qualities of meats so preserved, must be made. The requirement that a given chemical shall inhibit growth of a number of different fungi and also be safe for human consumption is difficult of fulfillment and is one which has imposed severe limitations upon the types of chemicals that may be employed. Obviously most of the antifungal materials used in plant disease control or in mildew-proofing of textiles must be eliminated from consideration.

MATERIALS AND METHODS

The molds used in these experiments were isolated from meats and meat products in cold storage at Duke University. Seventeen isolates, belonging to eleven species, were studied. These are as follows:

Penicillium crustosum Thom (7)*
Penicillium cyaneum Bainier and Sartory (3)
Penicillium cyclopium Westling (2, 6, 18, 20)
Penicillium olivino-viride Biourge (1, 16, 17)
Penicillium waksmani Zaleski (19)
Aspergillus niger van Tieghem (8)
Aspergillus sydowi Bainier and Sartory (15)
Cephalosporium sp. (9)
Hormodendrum cladosporioides Bonorden (10)
Thamnidium chaetocladioides Link (11)

We are indebted to Dr. K. B. Raper, Northern Regional Research Laboratory, Peoria, Illinois, for identification of the cultures

* The numbers in parentheses were assigned to the isolates employed.

of *Penicillium*. Stock cultures of the test fungi were maintained on slants of Czapek's agar, at room temperature, and transferred at intervals of 10–14 days.

Two different media were employed in the testing of fungistatic agents. One was Czapek's solution, consisting of $NaNO_3$, 3.0 gm.; KH_2PO_4, 1.0 gm.; $MgSO_4$, 0.5 gm.; KCl, 0.5 gm.; $FeCl_3$, trace; and sucrose, 30.0 gm. per liter. The other medium was devised in an attempt to simulate the conditions which obtain in fresh meats, the nitrogen being supplied as glutamic acid and the pH adjusted to 6.0. This medium contained glutamic acid, 2.0 gm.; KH_2PO_4, 0.3 gm.; K_2HPO_4, 2.7 gm.; $MgSO_4$, 0.5 gm.; and dextrose, 50.0 gm. per liter. The two media thus differ in pH, in nitrogen source, in carbon source, and in buffer capacity.

Since the primary consideration in meat preservation is the prevention of spoilage consequent upon growth of fungi, fungistatic rather than fungicidal techniques were chosen.

In testing, the fungi were grown in 125 ml. Erlenmeyer flasks containing 50 ml. of medium, supplemented with the various antifungal materials. Controls provided in each instance consisted of the basal media without added inhibitory chemicals. Except as otherwise indicated, the chemicals were added to the basal media and sterilized by autoclaving 15 minutes at 15 lb. pressure. The pH of the media (4.5 for Czapek's solution and 6.0 for the glutamic acid basal medium) was not adjusted after addition of the inhibitory materials.

Growth of the molds was recorded after one and two weeks' incubation at room temperature, using an arbitrary scale of 0, +, ++, +++, and ++++ in comparison with appropriate controls. For conciseness, only data from observations after two weeks are included herein. Concentrations of fungistatic agents are expressed in terms of percentage on a weight basis, in the case of solids, and on a volume basis for liquid materials.

The findings in the present study are based upon a total of over 9000 cultures.

LITERATURE SURVEY OF CHEMICAL AGENTS

The present experiments include tests of 31 different chemicals. No claims are made that all are safe for human consumption, but,

insofar as information is available, this consideration governed the choice of materials to be tested.

It was shown by Kiesel (**1913**), using *Aspergillus niger*, that acetic acid is quite toxic to fungi. Schönberg (**1943**) states that the Germans extensively employed acetic acid in meat preservation during World War II, and his experiments indicate that exposure for 10 minutes to 1.5–2.0% acetic acid will kill spores of *Penicillium glaucum*, *Aspergillus niger*, *Cladosporium herbarum* and *Mucor racemosus*. Levine and Fellers (**1940**) studied the influence of pH upon toxicity of acetic acid to *Aspergillus niger*. Sodium acetate was used for purposes of comparison. Sodium diacetate, $(CH_3COONa)_x \cdot (H_2O)_x$, a double salt of sodium acetate and acetic acid, was tested herein because of previous work of Glabe (**1942**), who employed it for prevention of mold and ropeformation in bakery products.

Considerable attention has been devoted to propionic acid and its salts upon growth of fungi. Kiesel (**1913**) reported that 0.0525–0.0586% propionic acid is toxic to *Aspergillus niger*. According to Desgrez and Saggio (**1907**), this acid is relatively nontoxic to animals, the lethal dose for rabbits by the intravenous route being 450 mg. per kilo. Peck and Rosenfeld (**1938**) report that growth of *Trichophyton gypseum*, *Epidermophyton inguinale*, and *Candida albicans* in Sabouraud's broth is inhibited by propionic acid in concentrations of 0.03%, 0.1%, and 1.0% respectively. Hoffman, Schweitzer and Dalby (**1939**) studied the influence of pH on the toxicity of propionic acid to fungi. Olson and Macy (**1940, 1946**) found that propionic acid is far more fungistatic than the sodium or calcium salts and ascribed fungistasis to the undissociated propionic acid molecule.

Calcium propionate is commonly incorporated with bread dough to inhibit growth of molds. In 1942, more than 1600 bakeries were employing propionate, and the number of loaves so treated was expected to exceed two billion (Miller, **1942**). Propionates are also employed to retard molding of cheese (Miller, **1940**), and, by treatment of wrapping papers, to prevent moldiness of butter (Macy and Olson, **1939**).

It was shown by Keeney (**1943**) and Keeney, Ajello, and Lank-

ford (**1944**) that sodium propionate inhibits growth of a large number of human pathogens, the amounts required varying from 0.0125 to 1.25% for the different ones. Satisfactory therapeutic results with 10% sodium propionate in the treatment of tinea pedis, tinea cruris, tinea capitis, thrush, and otomycosis have been reported by Keeney and Broyles (**1943**). Alter, Jones and Carter (**1947**) successfully employed propionates in the treatment of mycotic vulvovaginitis.

Harshbarger (**1942**) fed sodium and calcium propionate to rats at levels corresponding to 1% and 3% of the total food intake, without ill effects. Rowntree (Keeney, **1943**) injected 20 mg. of sodium propionate into rats daily for nearly a year without untoward results. Keeney, Ajello and Lankford (**1944**) found that the highest non-toxic daily dose of sodium propionate to mice by the intraperitoneal route is 5 mg. Walker and Coppock (**1928**) were able to cultivate *Aspergillus niger* on a medium containing calcium propionate as the sole source of carbon, a finding which is of considerable interest in connection with the use of propionates to check mold growth.

Our tests involved, among the higher fatty acids, caprylic acid, undecylenic acid, and their sodium salts. Peck and Rosenfeld (**1938**) reported that 0.03% caprylic acid is inhibitory to *Trichophyton gypseum*. Wyss, Ludwig and Joiner (**1945**) determined inhibitory concentrations of caprylic acid for *Aspergillus niger*, *Trichophyton interdigitale* and *T. rubrum*. Foley, Herrmann, and Lee (**1947**) studied the influence of pH upon the inhibition by caprylic acid of *T. gypseum*. Sodium caprylate has been successfully used in the treatment of dermatophytosis of the feet (Keeney, Ajello, Lankford, and Mary, **1945**).

The antifungal activity of undecylenic acid has been much studied in recent years, notably by Peck and Rosenfeld (**1938**), using *Trichophyton gypseum*, Rogler and Greathouse (**1940**), using *Phymatotrichum omnivorum*, Wyss, Ludwig and Joiner (**1945**), using a number of saprophytic molds and human pathogens, Foley, Herrmann and Lee (**1947**), using *T. gypseum* and Rothman *et al.* (**1947**), using *Microsporum audouini*. Extensive use has been made of powders and ointments containing undecylenic acid in the

treatment of dermatophytosis. Satisfactory therapeutic results have been obtained by several investigators, including Keeney, Ajello, Broyles and Lankford (**1944**), Shapiro and Rothman (**1945**), Hopkins *et al.* (**1946**), and Sulzberger and Kanof (**1947**).

Some of the derivatives of benzoic acid have been studied also, since sodium benzoate was used in meat preservation by our armed forces during World War II (Jensen, **1942**). Perry and Beal (**1920**) reported that *Penicillium glaucum* failed to grow in the presence of 0.25% sodium benzoate. The toxicity of sodium benzoate to rats was tested by Harshbarger (**1942**) who found it toxic at the 3% level but not toxic when fed at the rate of 1% of the total diet.

p-Amino benzoic acid was shown to be fungistatic by Hoffman, Schweitzer and Dalby (**1942**). Its effects upon the growth of *Aspergillus niger*, *Penicillium roqueforti* and *Byssochlamys fulva* under varying conditions of pH and nitrogen supply have recently been investigated by Cavill and Vincent (**1945, 1948**). p-Hydroxy benzoic acid was noted by Uppal (**1926**) to be toxic to *Phytophthora colocasiae*, and it was studied in comparison with the methyl and butyl esters of this acid. The esters of p-hydroxy benzoic acid, according to Neidig and Burrell (**1944**) and Jacobs (**1944**), were used as preservatives of meat products and other foodstuffs in Germany, and are employed by geneticists in Drosophila cultures to suppress mold growth. The methyl and ethyl esters have been used for treatment of dermatophytosis by Bang (**1937**) and Holm (**1944**). The toxicity of these esters to animals is extremely low, the lethal dose being of the order of 3–6 gm. per kilo (Neidig and Burrell, **1944**).

Myers (**1927**) found that thymol, carvacrol, and a number of essential oils, including oil of cloves, are fungicidal. Kingery and Adkisson (**1928**) reported thymol 1:7500 to be fungistatic to a number of dermatophytes, to *Sporotrichum schenckii* and to *Coccidioides immitis*. Our experiments therefore included thymol, carvacrol, oil of cloves, and "Stabilizer No. 1," the latter a mixture of mono isopropyl meta and para cresols, intended for use as a mold inhibitor for starches used in the sizing of paper. A study

of oral toxicity of "Stabilizer No. 1" with experimental animals indicated an LD_{50} of 1.2 gm. per kilo (Horsey, **1948**).

Three quinone derivatives were tested, among them, 2, 3, 5, 6 tetrachloro 1, 4 benzoquinone, or "Spergon," an agricultural fungicide used to protect germinating seeds of various crop plants from fungus attack. The LD_{50} of this compound for rats by the oral route was found to be 4 gm. per kilo (McGavack, Boyd, Terranova, and Lehr, **1943**). McNew (**1948**), studying the toxicity to swine of corn treated with tetrabenzoquinone, found that treated seed did not adversely affect animals even when they were maintained on it as long as 117 days. The antifungal activity of 2, 3 dichloro 1, 4 naphthoquinone against 22 species of fungi was investigated by Ter Horst and Felix (**1943**). Woolley (**1945**) tested the effect of this compound upon the growth of bacteria, *Saccharomyces cerevisiae* and *Endomyces vernalis*. Tests made by workers at the New York Medical College in 1943 disclose an LD_{50} to albino rats by the oral route of 3.5 gm. per kilo. Colwell and McCall (**1946**) noted that 2 methyl 1, 4 naphthoquinone is toxic to *Aspergillus niger*. Studies of its toxicity to experimental animals were made by Molitor and Robinson (**1940**).

Various acids, including boric, malonic, crotonic, sorbic and salicylic acids, have been examined in the present study. Boric acid is among the preservatives used in the control of molds of meats by our armed forces during World War II, according to Jensen (**1943**). Malonic acid, which suppresses respiration through its action upon the enzyme succinic dehydrogenase, was included because of studies on *Neurospora* by Ryan, Tatum and Giese (**1944**). Gooding (**1945**) suggested the use of crotonic acid or sorbic acid as a mold inhibitor in foods. Salicylic acid is well known as an antifungal material, and is used in treatment of superficial fungus infections.

Sodium nitrite, an ingredient used in solutions for curing meats, is the only component of such solutions with appreciable antifungal activity.

The compound 2, 4, 5 trichlorophenyl acetate is an agricultural fungicide for use to control cotton anthracnose fungus, *Glomerella gossypii*, on cotton seed. Its LD_{50} for mice is approximately 1.3

gm. per kilo, and this material did not interfere with growth of mice when added to the diet at the rate of 2% (Horsey, **1948**).

"G-4" is 2, 2'dihydroxy 5, 5'dichloro diphenyl methane, and is used in the mildewproofing of canvas, tenting, and other fabrics. Hexamethylene tetramine, according to Jacobs (**1944**), has been employed medically in the treatment of urinary tract infections, and in Germany is a permissible food preservative.

RESULTS

The results, as presented in tables I–VIII, give the lowest concentration of the various chemical agents which inhibit growth of the test fungi. When one or more organisms were suppressed by the lowest of a series of concentrations tested, the result is reported as less than the concentration stated. Similarly, when one or more molds grew in the highest concentration tested, the result is expressed as more than the stated concentration. Limits were imposed in some cases by the solubility of the chemical agents. From

TABLE I

PERCENTAGE CONCENTRATIONS OF ACETIC ACID AND CERTAIN SALTS REQUIRED FOR FUNGISTASIS

Molds	Concentration				
	Acetic acid		Sodium acetate	Sodium diacetate	
	Czapek	Glutamic	Czapek	Czapek	Glutamic
P. crustosum (7)	0.2	0.25	18	<0.5	0.75
P. cyaneum (3)	0.15	<0.15	15	<0.5	<0.5
P. cyclopium (2)	0.2	0.25	18	<0.5	0.75
P. cyclopium (6)	0.2	0.25	18	<0.5	0.75
P. cyclopium (18)	0.2	0.25	>18	0.75	0.75
P. cyclopium (20)	0.2	0.25	18	0.75	0.75
P. martensii (4)	0.15	<0.15	18	<0.5	<0.5
P. martensii (12)	0.15	0.25	18	<0.5	<0.5
P. olivino-viride (1)	0.15	0.25	18	0.75	0.75
P. olivino-viride (16)	0.15	0.25	>18	0.75	0.75
P. olivino-viride (17)	0.15	0.2	15	<0.5	<0.5
P. waksmani (19)	<0.1	0.25	18	<0.5	<0.5
A. niger (8)	0.2	0.25	18	<0.5	0.75
A. sydowi (15)	<0.1	0.25	>18	0.75	0.75
Cephalosporium sp. (9)	<0.1	<0.15	15	<0.5	<0.5
H. cladosporioides (10)	<0.1	<0.15	9	<0.5	<0.5
T. chaetocladioides (11)	0.2	0.25	9	0.75	0.75

a practical standpoint, special significance is attached to the lowest concentration inhibiting growth of all the test organisms.

Acetic acid (TABLE I) inhibits growth of all the fungi tested in a concentration of 0.2% on Czapek's medium, and 0.25% on the glutamic acid medium. For comparative purposes, tests were made with sodium acetate. None of the molds was inhibited by concentrations of less than 9% sodium acetate, and three were not inhibited by 18%. These findings indicate that the acetate ion is

TABLE II

INHIBITION OF MEAT MOLDS BY PROPIONIC ACID AND ITS SALTS

Molds	Concentration					
	Propionic acid		Sodium propionate		Sodium propionate	Calcium propionate
	Czapek	Glutamic	Czapek	Glutamic	Czapek sugar	Czapek
P. crustosum (7)	0.15	0.2	12	7	8	5
P. cyaneum (3)	<0.1	0.15	4	3	6	3
P. cyclopium (2)	0.15	0.2	10	7	12	>6
P. cyclopium (6)	0.15	0.2	12	7	12	3
P. cyclopium (18)	0.15	0.2	12	5	>12	>6
P. cyclopium (20)	0.15	0.2	12	7	12	>6
P. martensii (4)	<0.1	0.15	10	5	10	3
P. martensii (12)	<0.1	0.15	10	5	8	>6
P. olivino-viride (1)	<0.1	0.15	10	5	8	>6
P. olivino-viride (16)	<0.1	0.15	10	5	>12	>6
P. olivino-viride (17)	0.15	0.15	12	5	10	>6
P. waksmani (19)	<0.1	0.15	10	5	8	3
A. niger (8)	<0.1	0.15	4	5	6	3
A. sydowi (15)	<0.1	0.15	10	5	12	5
Cephalosporium sp. (9)	<0.1	<0.1	4	3	6	<1
H. cladosporioides (10)	<0.1	<0.1	4	3	6	<1
T. chaetocladioides (11)	<0.1	0.15	6	5	>2	<1

virtually non-toxic to these fungi, and that a large part of the toxicity of acetic acid may be ascribed to the non-ionized acetic acid molecule.

The sample of sodium diacetate tested was stated by the manufacturer to have an acetic acid content of 33–35%. This agent was inhibitory to all molds in a concentration of 0.75%. Comparison of this result with those obtained with acetic acid and with sodium acetate indicates that the inhibitory action of sodium diacetate is due to its acetic acid content.

Propionic acid (TABLE II) is slightly more toxic to molds than is acetic acid, concentrations inhibitory to all organisms being 0.15% on Czapek's medium and 0.2% on glutamic acid medium. The molds used in the present work are far more resistant to propionates than are the human pathogens. A concentration of 4% sodium propionate was required to inhibit the more sensitive meat molds on Czapek's medium, while the concentration had to be increased to 12% to inhibit the more resistant organisms. Sodium propionate was found to be more effective on the glutamic acid medium than on Czapek's medium, a concentration of 7% sodium propionate checking all mold growth on the glutamic acid medium. These results, apparently anomalous in relation to the initial pH of the two basal media without added propionate, may be interpreted as due to a shift in pH of the media owing to the addition of an "alkaline" salt, and its effect upon the ionization constant of propionic acid. Calcium propionate proved more toxic to the test fungi than did sodium propionate. The calcium salt is, however, so insoluble that a 6% concentration, approaching saturation, was insufficient to check growth of seven of the test organisms.

In attempting to evaluate the significance of propionates as a carbon source for fungi in relation to fungistasis, Czapek's medium, lacking sugar, was prepared and varying amounts of sodium propionate were added as a sole source of carbon. Resultant growth indicated that all of the molds, with the single exception of *Thamnidium chaetocladioides*, are able to utilize propionate. Sodium propionate is far inferior to sucrose as a carbon source for these fungi, however, since growth of the +++ and ++++ categories was not obtained in any instance upon propionate, regardless of its concentration. The inhibitory concentrations of sodium propionate in Czapek's medium lacking sucrose compare favorably with those obtained in the same medium containing sucrose. The fact that fungi are able to utilize propionate as a carbon source appears to offer at least a partial explanation of the large amounts required to inhibit their growth.

Caprylic acid (TABLE III) is more toxic to meat molds than the fatty acids of lower molecular weight, the concentrations required for suppression of all growth being 0.05% on Czapek's medium

TABLE III

CONCENTRATIONS OF CAPRYLIC ACID AND UNDECYLENIC ACID, AND THEIR SALTS REQUIRED FOR FUNGISTASIS OF MEAT MOLDS

Molds	Concentration							
	Caprylic acid		Sodium caprylate		Undecylenic acid		Sodium undecylenate	
	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	0.025	0.075	0.3	0.2	>0.2	0.05	0.05	0.1
P. cyaneum (3)	<0.01	0.05	<0.05	0.1	0.05	0.025	<0.025	<0.025
P. cyclopium (2)	0.05	0.075	0.3	>0.3	>0.2	0.05	0.1	0.1
P. cyclopium (6)	0.05	0.075	0.3	0.3	>0.2	0.1	0.1	0.1
P. cyclopium (18)	0.025	0.075	0.3	0.3	>0.2	0.05	0.1	0.1
P. cyclopium (20)	0.025	0.075	0.3	0.3	>0.2	0.05	0.1	0.1
P. martensii (4)	0.025	0.05	0.3	0.2	0.2	0.05	0.05	0.05
P. martensii (12)	0.05	0.075	0.1	0.2	>0.2	0.05	0.1	0.1
P. olivino-viride (1)	0.05	0.05	0.3	0.3	>0.2	0.025	0.1	0.05
P. olivino-viride (16)	0.025	0.075	0.1	0.2	>0.2	0.05	0.05	0.05
P. olivino-viride (17)	0.025	0.075	0.3	0.2	>0.2	0.05	0.05	0.1
P. waksmani (19)	0.05	0.075	0.3	0.3	>0.2	0.05	0.05	0.1
A. niger (8)	0.05	0.075	0.3	0.3	>0.2	0.05	0.1	0.05
A. sydowi (15)	0.05	0.075	0.05	0.3	0.05	0.05	0.1	0.1
Cephalosporium sp. (9)	<0.01	<0.025	<0.05	<0.05	0.05	0.025	<0.025	<0.025
H. cladosporioides (10)	<0.01	<0.025	<0.05	<0.05	<0.01	0.025	<0.025	<0.025
T. chaetocladioides (11)	0.025	0.075	0.1	0.1	>0.2	>0.1	0.1	0.1

and 0.075% on the glutamic acid medium. Tests with sodium caprylate gave a value of 0.3% on both media.

Experiments with undecylenic acid in Czapek's medium gave no satisfactory mold control at any concentration tested, a result attributable to the very low solubility of this material in water under conditions of low pH. With this acid in the glutamic acid medium, growth of all except two of the test organisms was checked at a concentration of 0.05%. Sodium undecylenate added in the amount of 0.1% inhibited all fungi on both media.

A concentration of 1.5% sodium benzoate (TABLE IV) was required to suppress growth of all the meat molds, so that this material is a rather poor fungistatic agent. Using p-amino benzoic acid, 0.5% was required on Czapek's medium and 1% on glutamic acid medium. p-Hydroxy benzoic acid was ineffective against certain of the molds at a concentration of 1.5%, the limit of its solubility. The esters of p-hydroxy benzoic acid, however, are efficient fungistatic agents, the methyl ester being inhibitory to all the molds tested on both media at a concentration of 0.1%. The butyl ester is the more toxic, but is sufficiently insoluble so that a few of the molds can grow in the presence of 0.02% p-hydroxy n-butyl benzoate, a concentration which represents the limit of solubility of this compound.

There is little difference in the effectiveness of thymol, carvacrol, "Stabilizer No. 1" and oil of cloves (TABLE V). All are effective in the range of concentrations of 0.025–0.05%.

The three quinone derivatives, tetrachlorobenzoquinone, dichloronaphthoquinone, and 2-methyl naphthoquinone, are not heat stable and so were sterilized by Berkefeld filtration. Because these compounds are only sparingly soluble in water, they were dissolved in minimal quantities of 95% alcohol. Tetrachlorobenzoquinone (TABLE VI) was found inhibitory to growth of all except three test organisms at a concentration of 0.005% in Czapek's medium and 0.01% in the glutamic acid medium. Essentially similar findings were obtained with dichloronaphthoquinone. Mold control was obtained with 0.01% 2- methyl naphthoquinone in Czapek's medium, but this concentration, the limit of its solubility, failed to inhibit most of the meat molds on the glutamic acid medium.

TABLE IV

CONCENTRATION OF BENZOATES AND FUNGISTASIS OF MOLDS ON MEATS

Molds	Concentration								
	Sodium benzoate	p-Amino benzoic acid		p-Hydroxy benzoic acid		p-Hydroxy methyl benzoate		p-Hydroxy n-butyl benzoate	
	Czapek	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	1.5	0.5	1.0	1.25	>1.5	0.1	<0.05	>0.02	0.02
P. cyaneum (3)	1.25	0.25	0.75	1.25	>1.5	0.05	<0.05	0.02	<0.01
P. cyclopium (2)	1.5	0.5	1.0	1.25	>1.5	0.05	0.1	0.02	0.02
P. cyclopium (6)	1.5	0.5	1.0	1.25	>1.5	0.05	0.1	>0.02	0.02
P. cyclopium (18)	1.5	0.5	1.0	1.25	>1.5	0.1	0.1	0.02	0.02
P. cyclopium (20)	1.5	0.5	1.0	1.25	>1.5	0.1	0.1	>0.02	0.02
P. martensii (4)	1.0	0.5	1.0	1.25	>1.5	0.1	0.1	0.02	>0.02
P. martensii (12)	1.25	0.5	0.75	1.25	>1.5	0.1	0.05	0.02	>0.02
P. olivino-viride (1)	1.5	0.25	<0.5	1.25	>1.5	0.05	<0.05	<0.01	<0.01
P. olivino-viride (16)	1.5	0.5	<0.5	1.25	1.5	0.05	<0.05	<0.01	0.01
P. olivino-viride (17)	1.5	0.25	1.75	1.25	>1.5	0.05	<0.05	<0.01	<0.01
P. waksmani (19)	1.5	0.25	0.75	>1.5	>1.5	0.1	0.1	>0.02	0.02
A. niger (8)	1.5	0.5	0.75	>1.5	>1.5	0.1	0.1	>0.02	0.02
A. sydowi (15)	1.25	0.25	1.0	>1.5	>1.5	0.05	0.01	<0.01	0.02
Cephalosporium sp. (9)	<0.5	0.25	<0.5	<0.5	<0.5	0.5	0.05	>0.02	0.02
H. cladosporioides (10)	<0.5	<0.1	<0.5	<0.5	<0.5	0.05	0.05	0.02	<0.01
T. chaetocladioides (11)	1.0	0.5	1.0	1.25	>1.5	0.1	0.1	<0.01	<0.01

TABLE V

INHIBITION OF MEAT MOLDS BY THYMOL AND RELATED OILS

Molds	Concentration							
	Thymol		Carvacrol		Stabilizer No. 1		Oil of cloves	
	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	0.02	<0.025	<0.025	0.05	0.02	0.05	0.05	0.05
P. cyaneum (3)	<0.01	0.05	<0.025	<0.025	0.035	<0.035	0.05	0.05
P. cyclopium (2)	0.02	0.05	>0.05	0.05	0.035	0.05	0.05	0.05
P. cyclopium (6)	0.035	0.05	0.05	0.05	0.035	0.05	0.05	0.05
P. cyclopium (18)	0.035	0.05	0.05	0.05	0.035	0.05	0.05	0.05
P. cyclopium (20)	0.035	0.05	0.05	0.05	0.035	0.05	0.05	0.05
P. martensii (4)	0.025	0.05	>0.05	0.05	0.025	0.05	0.05	0.05
P. martensii (12)	0.025	0.05	<0.025	0.05	0.02	0.05	0.05	0.05
P. olivino-viride (1)	0.02	0.05	0.05	0.05	0.035	<0.035	0.05	<0.025
P. olivino-viride (16)	0.02	0.05	<0.025	<0.025	0.02	<0.035	0.05	0.05
P. olivino-viride (17)	0.02	0.05	<0.025	0.05	0.025	0.05	0.05	<0.025
P. waksmani (19)	0.02	0.05	0.05	0.05	0.035	0.05	0.05	0.05
A. niger (8)	0.025	0.05	<0.025	0.05	0.035	0.05	0.05	0.05
A. sydowi (15)	<0.01	0.05	<0.025	<0.025	0.02	<0.035	0.05	0.05
Cephalosporium sp. (9)	0.02	0.05	<0.025	0.05	0.025	<0.035	0.05	0.05
H. cladosporioides (10)	<0.01	<0.025	<0.025	<0.025	0.02	<0.035	<0.025	0.05
T. chaetocladioides (11)	<0.01	<0.025	<0.025	<0.025	<0.01	<0.035	0.05	0.05

Boric acid (TABLE VII) is a weak fungistatic agent, as two of the test strains were not checked by 2% of this material. Malonic acid failed to check growth of many of the fungi when present in a concentration of 0.7%. Crotonic acid proved to be an effective fungistatic material, the concentrations required being 0.1% in Czapek's medium and 0.15% in glutamic acid medium. Its effectiveness is of the same order of magnitude as that of the closely related compound, sorbic acid, which suppressed growth of all molds in both media in a concentration of 0.1%. Salicylic acid has

TABLE VI

CONCENTRATION OF CERTAIN QUINONES AND INHIBITION OF MEAT MOLDS

Molds	Concentration					
	Tetrachloro-benzoquinone		Dichloro-naphthoquinone		2 Methyl naphthoquinone	
	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	0.001	0.01	<0.0005	0.005	0.01	>0.01
P. cyaneum (3)	0.001	0.01	0.001	0.01	0.01	>0.01
P. cyclopium (2)	0.005	0.01	<0.0005	0.005	0.005	>0.01
P. cyclopium (6)	0.005	0.01	<0.0005	0.005	0.01	>0.01
P. cyclopium (18)	0.005	0.01	0.005	0.01	0.01	0.01
P. cyclopium (20)	0.005	0.01	<0.0005	0.005	0.01	0.01
P. martensii (4)	0.001	0.01	0.005	0.01	0.01	>0.01
P. martensii (12)	0.005	0.01	0.001	0.005	0.01	>0.01
P. olivino-viride (1)	0.005	0.01	0.001	0.01	0.005	>0.01
P. olivino-viride (16)	0.005	0.01	0.0005	0.005	0.005	>0.01
P. olivino-viride (17)	0.005	0.01	0.001	0.005	0.005	>0.01
P. waksmani (19)	0.005	>0.01	0.001	0.01	0.01	>0.01
A. niger (8)	0.01	>0.01	<0.0005	0.01	0.01	0.005
A. sydowi (15)	0.005	>0.01	0.001	>0.01	0.01	0.005
Cephalosporium sp. (9)	0.005	0.01	<0.0005	0.005	<0.001	>0.01
H. cladosporioides (10)	0.005	0.01	<0.0005	0.005	0.005	0.005
T. chaetocladioides (11)	0.005	0.01	0.01	0.01	0.01	>0.01

slightly more fungistatic activity. A concentration of 0.05% in Czapek's medium and 0.1% in glutamic acid medium resulted in complete inhibition of growth.

Sodium nitrite (TABLE VIII), the only ingredient of solutions used to cure meats that has appreciable antifungal activity, gave good results at the 0.5% level in tests with Czapek's solution, but 2% was required in glutamic acid medium. Trichlorophenyl acetate proved inhibitory in tests on both media at a concentration of 0.005%. "G-4" prevented growth of the various test organisms at

TABLE VII

FUNGISTATIC CONCENTRATION OF CERTAIN ACIDS FOR MEAT MOLDS

Molds	Concentration									
	Boric acid		Malonic acid		Crotonic acid		Sorbic acid		Salicylic acid	
	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	1	<1.5	0.6	>0.7	0.05	0.15	0.1	0.075	0.05	0.1
P. cyaneum (3)	1.5	2	0.6	>0.7	<0.025	0.1	0.025	0.025	0.05	0.1
P. cyclopium (2)	2	2	>0.7	>0.7	0.05	0.1	0.1	0.075	0.05	0.1
P. cyclopium (6)	<1	<1.5	>0.7	>0.7	0.1	0.15	0.1	0.1	0.05	0.1
P. cyclopium (18)	<1	<1.5	0.6	>0.7	0.05	0.1	0.025	0.1	0.05	0.1
P. cyclopium (20)	2	<1.5	0.6	>0.7	0.1	0.15	0.05	0.1	0.05	0.1
P. martensii (4)	>2	2	0.4	>0.7	0.05	0.1	0.05	0.075	0.05	0.1
P. martensii (12)	>2	>2	0.4	>0.7	0.05	0.1	0.05	0.1	0.05	0.1
P. olivino-viride (1)	<1	<1.5	0.6	>0.7	0.05	0.1	0.1	0.075	0.05	0.1
P. olivino-viride (16)	1.5	<1.5	0.6	>0.7	0.05	0.1	0.025	0.075	0.05	0.1
P. olivino-viride (17)	<1	<1.5	0.4	>0.7	0.05	0.1	0.025	0.075	0.05	0.1
P. waksmani (19)	<1	<1.5	0.6	>0.7	0.05	0.1	0.1	0.1	0.05	0.1
A. niger (8)	2	<1.5	>0.7	>0.7	0.05	0.1	0.1	0.1	0.05	0.1
A. sydowi (15)	2	<1.5	>0.7	>0.7	0.1	0.15	0.1	0.1	0.05	0.1
Cephalosporium sp. (9)	<1	<1.5	0.4	0.4	<0.025	0.1	0.025	0.025	0.05	0.1
H. cladosporioides (10)	1.5	<1.5	<0.2	0.4	<0.025	<0.05	0.025	0.025	0.05	0.1
T. chaetocladioides (11)	<1	<1.5	<0.2	0.4	0.05	0.1	0.025	0.025	0.05	0.1

TABLE VIII

CONCENTRATION OF SODIUM NITRITE AND CERTAIN ORGANIC FUNGICIDES REQUIRED TO INHIBIT MEAT MOLDS

Molds	Concentration							
	Sodium nitrite		Trichlorophenyl Acetate		"G-4"		Hexamethylenetetramine	
	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic	Czapek	Glutamic
P. crustosum (7)	0.5	2	0.001	0.005	0.01	<0.005	0.1	<0.05
P. cyaneum (3)	0.1	1	0.005	0.005	>0.01	<0.005	<0.05	<0.05
P. cyclopium (2)	0.25	2	0.001	0.005	0.01	<0.005	0.1	<0.05
P. cyclopium (6)	>0.5	2	0.001	0.005	<0.005	<0.005	0.1	0.1
P. cyclopium (18)	0.5	1	0.001	0.005	>0.01	<0.005	0.15	<0.05
P. cyclopium (20)	0.5	1.5	0.001	0.005	0.01	<0.005	<0.05	<0.05
P. martensii (4)	0.1	0.5	0.001	0.005	0.01	<0.005	0.2	0.2
P. martensii (12)	0.1	1	0.001	>0.005	0.01	<0.005	>0.2	0.15
P. olivino-viride (1)	0.5	1	0.005	0.005	0.01	<0.005	<0.05	<0.05
P. olivino-viride (16)	0.5	2	>0.0005	0.005	>0.01	<0.005	0.15	0.1
P. olivino-viride (17)	0.25	0.5	0.001	0.005	0.01	<0.005	0.15	0.15
P. waksmani (19)	0.5	1	0.005	0.005	0.01	<0.005	<0.05	<0.05
A. niger (8)	0.25	1	0.001	>0.005	0.01	<0.005	<0.05	<0.05
A. sydowi (15)	0.5	1.5	0.005	0.005	0.01	<0.005	0.1	<0.05
Cephalosporium sp. (9)	0.5	0.5	0.001	0.005	0.01	<0.005	<0.05	<0.05
H. cladosporioides (10)	0.1	0.5	0.001	0.005	<0.005	<0.005	<0.05	<0.05
T. chaetocladioides (11)	0.5	2	0.005	0.005	0.01	<0.005	0.15	0.1

a concentration of 0.005–0.01%. Hexamethylenetetramine proved to be inhibitory at a level of 0.2%.

DISCUSSION

The problem of chemical control of molds on meats is complex, involving consideration of concentrations required to achieve fungistasis under different environmental conditions, toxicity to man, absence of appreciable odor or taste, methods of application, and cost. The present report constitutes a preliminary approach to the problem, and the data do not justify a complete answer to the question of which chemical agent is ideal for mold control. As a result of this screening procedure, however, certain of the chemicals tested appear to be well-suited, others may or may not be of value, and others may be eliminated from further consideration.

Acetic acid and sodium diacetate appear especially deserving of further consideration, whereas sodium acetate is obviously worthless. Propionic acid appears to be sufficiently effective as a fungistatic agent, but may be found undesirable because of taste or odor. Sodium and calcium propionates have several distinct disadvantages, notably the large amounts required for prevention of mold growth, the utilization of these compounds as sources of carbon for certain fungi, and the relative insolubility of the calcium salt. These materials, however, may merit further study. Likewise the higher fatty acids, caprylic acid and undecylenic acid, are effective fungistatic agents, although undecylenic acid has the disadvantage of low solubility in water, and both of these materials may be found objectionable because of taste or odor. These difficulties, however, are not encountered with sodium caprylate and sodium undecylenate, which should be considered further.

Although some use was made of sodium benzoate under World War II conditions and its fungistatic activity is much greater than that of the propionates, yet it does not appear to have sufficient antifungal activity to be of marked value. The same is true of p-amino benzoic acid. p-Hydroxybenzoic acid is obviously worthless for use against fungi, but its esters appear to have a number of favorable characteristics, and should definitely be considered for commercial use.

Each of the closely related materials, thymol, carvacrol, "Stabilizer No. 1," and oil of cloves, possesses marked fungistatic activity. Carvacrol and "Stabilizer No. 1," however, should probably be eliminated from further consideration because of their pronounced cresol odor. But thymol and oil of cloves should be tested more thoroughly from the standpoint of toxicity to man before attempts are made to employ them in meat preservation.

Tetrachlorobenzoquinone and dichloronaphthoquinone are sufficiently effective against fungi and nontoxic to man to merit further consideration. A serious disadvantage possessed by 2-methyl naphthoquinone is its sensitivity to light.

Boric and malonic acids do not appear to be sufficiently active against molds to be of appreciable value, notwithstanding the limited success obtained with boric acid during World War II. Crotonic and sorbic acids gave excellent results in the present experiments, and should be given further trials. It is felt that salicylic acid, while effective as an antifungal agent, should be further studied from a pharmacological standpoint.

Sodium nitrite does not possess sufficient fungistatic power to make it useful in preventing moldiness. Trichlorophenyl acetate appears to be an excellent inhibitor of fungus growth, while "G-4," like 2-methyl naphthoquinone, is inactivated in the presence of light. Hexamethylene tetramine may be worthy of further trial.

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SUMMARY

These experiments constitute attempts to evaluate 31 chemical agents for the control of mold spoilage of meats. The test fungi include *Penicillium crustosum, P. cyaneum, P. cyclopium, P. martensii, P. olivino-viride, P. waksmani, Aspergillus niger, A. sydowi, Cephalosporium* sp., *Hormodendrum cladosporioides* and *Thamnidium chaetocladioides.* The fungistatic concentration of each chemical agent has been determined for each test organism upon two different media.

It is concluded that acetic acid, sodium diacetate, sodium caprylate, sodium undecylenate, methyl p-hydroxy benzoate, n-butyl p-hydroxy benzoate, thymol, oil of cloves, tetrachlorobenzoquinone, dichloronaphthoquinone, crotonic acid, sorbic acid, and trichlorophenyl acetate possess greatest merit among the antifungal materials tested.

Propionic acid, sodium propionate, calcium propionate, caprylic acid, undecylenic acid, sodium benzoate, p-amino benzoic acid, carvacrol, "Stabilizer No. 1," salicylic acid, "G-4," and hexamethylene tetramine, for one reason or other, are not ideally adapted for control of molds on meats. Sodium acetate, p-hydroxy benzoic acid, 2-methyl naphthoquinone, boric acid, malonic acid, and sodium nitrite, however, appear worthless as fungistatic agents against molds on meats.

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A DACTYLELLA WITH CONIDIA RESEMBLING THOSE OF DACTYLELLA STENOBROCHA IN SIZE AND SHAPE

CHARLES DRECHSLER[1]

(WITH 1 FIGURE)

Several maizemeal-agar plate cultures, which, after being overgrown with mycelium of *Pythium mamillatum* Meurs, had been further planted with small quantities of leaf mold collected in deciduous woods near Newport News, Virginia, on October 15, 1948, became abundantly infested in about ten days with free-living nematodes as well as with different species of *Amoebae* and testaceous rhizopods. In all the cultures the eelworms then began to suffer losses from the capture and destruction of many individuals by the predacious hyphomycete I described earlier (2: 508–513) as *Dactylella gephyropaga*. After this hyphomycete had extended its mycelium throughout the substratum it continued for weeks to destroy eelworms and to produce conidia on its sparsely scattered conidiophores. Two of the cultures on being examined 35 days after the leaf mold had been added showed, besides, in areas near the deposits of forest detritus some development of erect conidiophores that bore solitary conidia manifestly alien not only to *Dactylella gephyropaga* but also to all congeneric species hitherto made known. During the ensuing weeks the new fungus gradually spread over both of the aging cultures, putting forth additional conidial apparatus rather sparingly though in ample quantity for study.

The colorless procumbent mycelial filaments (FIG. 1, *A–D*: *a*) from which the erect conidiophores (FIG. 1, *A–D*: *b*) of the new

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fungus arose were of moderate width for members of the predacious series, and like the hyphae of allied forms were divided at moderate intervals by commonplace cross-walls. The conidiophores similarly offered little distinctiveness. As their height did not usually exceed 400 μ, they appeared in general a little shorter than the intermixed conidiophores of *Dactylella gephyropaga*. From a comparison of measurements they would seem, perhaps, on the whole, somewhat shorter also than the conidiophores of my *Dactylella stenobrocha* (**5**) and my *Dactylella heterospora* (**3**), though the dimensional differences concerned here are not impressive for descriptive purposes. While the conidiophores of the new fungus, as a rule, were simple (FIG. 1, *A–C: b*) and regularly produced a single conidium (FIG. 1, *A, c*) at the tip, in occasional instances (FIG. 1, *D, b*) they gave off a lateral branch (FIG. 1, *D, c*) from which a second conidium would be abjointed. When eventually a denuded conidiophore fell onto the substratum it would frequently give rise to a secondary conidiophore from one of its living segments. Through repetition of the same developmental sequence conidiophores of the third and fourth orders were brought into being.

The conidia thus formed aloft sparingly were mostly of an elongated ellipsoidal or strobiliform shape, thereby showing rather marked similarity to the conidia of *Dactylella stenobrocha*, which, indeed, they further resembled closely in their main dimensions. With respect to shape and dimensions they bore notable resemblance also to the larger type of conidia produced by *Dactylella heterospora*, though they lacked the abruptly truncate basal profile so characteristic of the latter. They were decisively distinguished, however, from the conidia of both *D. stenobrocha* and *D. heterospora* by more abundant septation, as they contained from 1 to 9 cross-walls. The specimens showing one septum (FIG. 1, *E, a, b*), two septa (FIG. 1, *F, a–f*), and three septa (FIG. 1, *G, a–h*)—the entire range of septation in *D. stenobrocha*—included only about one-seventh of the conidia produced by the new fungus. Conidia with four (FIG. 1, *H, a–l*), five (FIG. 1, *I, a–j*), six (FIG. 1, *J, a–l*), or seven (FIG. 1, *K, a–p*) cross-walls were present in large numbers, while specimens with eight (FIG. 1, *L, a–b*), or nine

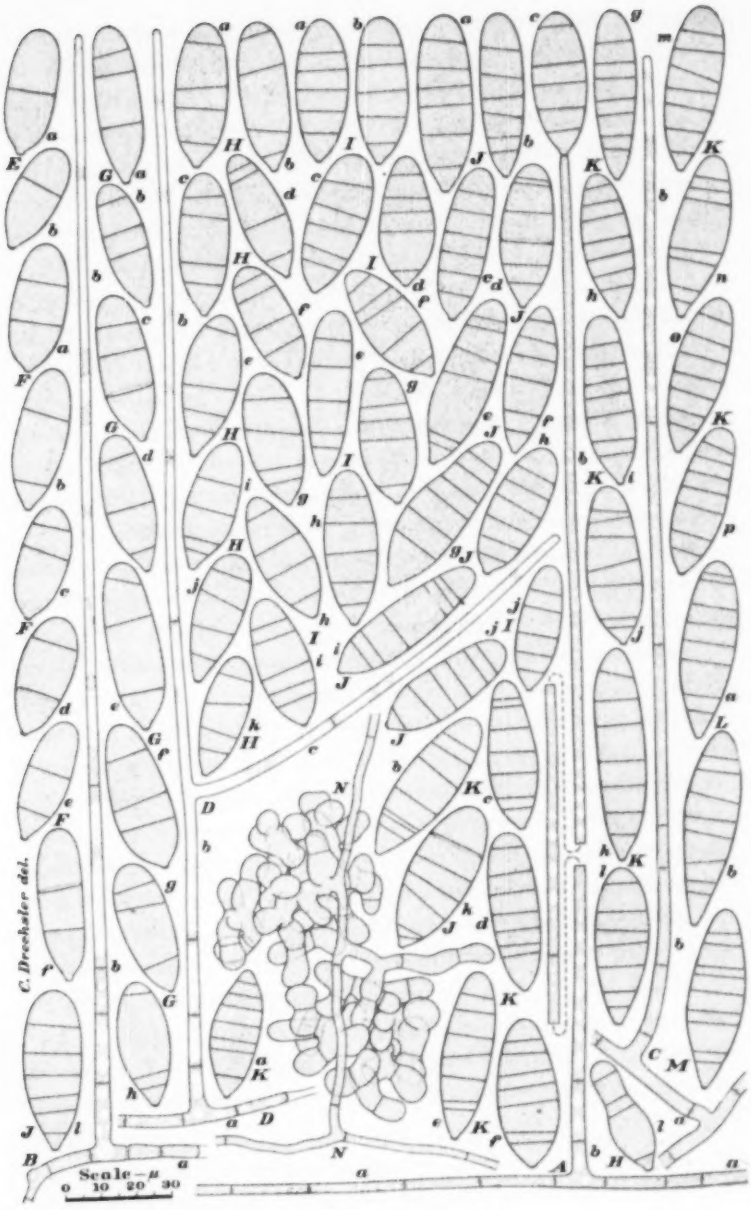

FIG. 1. *Dactylella strobilodes.*

(FIG. 1, *M*) partitions were few. Of 125 conidia taken more or less at random a frequency distribution was noted as follows: 1 septum, 2; 2 septa, 7; 3 septa, 9; 4 septa, 28; 5 septa, 29; 6 septa, 30; 7 septa, 17; 8 septa, 2; 9 septa, 1. The same assortment of conidia gave measurements for length, expressed in the nearest integral number of microns, distributed as follows: 31 μ, 1; 32 μ, 1; 34 μ, 2; 35 μ, 5; 36 μ, 6; 37 μ, 7; 38 μ, 14; 39 μ, 6; 40 μ, 17; 41 μ, 5; 42 μ, 14; 43 μ, 4; 44 μ, 13; 45 μ, 5; 46 μ, 8; 47 μ, 3; 48 μ, 5; 49 μ, 2; 50 μ, 4; 52 μ, 1; 55 μ, 1; 60 μ, 1; and measurements for greatest width, expressed in the nearest integral number of microns, with the following distribution: 11 μ, 1; 12 μ, 6; 13 μ, 15; 14 μ, 34; 15 μ, 33; 16 μ, 27; 17 μ, 7; 18 μ, 2.

Several attempts were made to isolate the fungus by removing its conidia from the erect conidiophores to newly poured plates of maizemeal agar—the removal being accomplished, with suitable precautions against contamination, by means of small slabs of sterile agar medium held on a flamed platinum spatula. The conidia in all instances failed to germinate on the sterile agar, though the same materials and procedures have been employed successfully in isolating nearly all of the clampless nematode-capturing hyphomycetes encountered under similar conditions.

As the general habit and morphology of its conidial apparatus left little room for doubt that the fungus was intimately related to the nematode-capturing hyphomycetes, its mycelium both on and under the surface of the agar was examined for some sort of biological relationship. However, I was unable to discover the fungus in any parasitic or predacious relationship either to eelworms or to any other of the several types of minute animals infesting the cultures. Nor could I find it parasitizing *Pythium mamillatum*, or *Dactylella gephyropaga*, or any other fungus that had grown out from the superadded leaf mold into the transparent agar. In a few places submerged filaments were found bearing a somewhat elaborate system of distended, colorless, septate, closely ramified hyphal elements, filled with protoplasm of nearly homogeneous consistency (FIG. 1, *N*). These ramifying systems had much resemblance to the dichotomously branched assimilative apparatus produced by *Dactylella tylopaga* Drechsl. (1) in captured speci-

mens of *Amoeba verrucosa* Ehrenb., but were never found loosely enveloped by any recognizable membrane. Hence there seems little likelihood that the fungus could have subsisted on a pelliculate rhizopod, though the possibility remains that it might have subsisted on a rhizopod devoid of a persistent pellicle. A destructive biological relationship involving a protozoan without any durable membrane—a relationship comparable to that of my *Acaulopage crobylospora* (**4**) to *Leptomyxa* sp.—might well be difficult of detection.

In respect to its uncertain biological character, the fungus invites comparison with the two allied species I referred earlier to *Dactylella rhombospora* Grove (**2**: 539–540) and *Dactylaria pulchra* Linder (**3**: 349–352), as also with the three species I have described under the binomials *Dactylella heptameres* (**3**: 352–354), *Dactylella rhopalota* (**3**: 354–357), and *Dactylella atractoides* (**3**: 357–360). Among the several species that have been described under *Dactylella* in the literature but have so far not developed in my agar cultures, it seems most to resemble *Dactylella minuta* Grove (**6**). Identity with *D. minuta* seems unlikely, however, as that species has considerably shorter (120 to 150 μ) conidiophores together with generally longer (60 to 70 μ) conidia; these conidia, moreover, to judge from Grove's figures, being of clavate rather than elongated ellipsoidal shape. The fungus from Newport News apparently has no close morphological similarity to any of the forms described in *Monacrosporium*, a genus presumably identical with *Dactylella*. Its description as a new species may advantageously forestall confusion with *Dactylella stenobrocha* and *Dactylella heterospora*. The specific epithet applied to it is intended to be conveniently suggestive in signalizing the resemblance in shape of its conidia to pine cones.

Dactylella strobilodes sp. nov.

Mycelium sparsum; hyphis sterilibus incoloratis, mediocriter septatis, plerumque 2–4.5 μ crassis, ex maxima parte parce ramosis, sed cristas crebre ramosas in ramulis brevibus 4–8.5 μ latis consistentes rarissime ferentibus; hyphis fertilibus incoloratis, erectis, plerumque 3–8 septatis, 250–400 μ altis, basi 4–7.5 μ crassis, sursum leniter attenuatis, apice 2–3 μ crassis, vulgo simplicibus sed quandoque ramo praeditis, in quoque apice conidium gignentibus; conidiis incoloratis, elongato-ellipsoideis vel strobiliformibus, apice late rotun-

datis, basi saepe pediculo obtuso praeditis, 1–9 septatis plerumque 3–7 septatis, 30–60 μ (saepius circa 41.5 μ) longis, 11–18 μ (saepius circa 14.6 μ) crassis. Habitat in humo silvestri prope Newport News, Virginia.

Mycelium sparse; vegetative hyphae colorless, septate at moderate intervals, mostly 2 to 4.5 μ wide, in general rather sparingly branched but in widely spaced positions bearing closely ramified systems composed of branches often 4 to 8.5 μ in diameter. Conidiophores colorless, erect, often containing 3 to 8 cross-walls, commonly 250 to 400 μ high, 4 to 7.5 μ wide at the base, narrowing gradually upward, mostly 2 to 3 μ wide at the tip, usually simple but occasionally with a lateral branch, at each tip bearing a single conidium. Conidia colorless, elongated ellipsoidal or strobiliform, broadly rounded at the tip, at the base often furnished with a blunt hilar protrusion, divided by 1 to 9 (usually by 3 to 7) cross-walls, measuring mostly 30 to 60 μ (average 41.5 μ) in length and 11 to 18 μ (average 14.6 μ) in width.

Occurring in leaf mold in deciduous woods near Newport News, Virginia.

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EXPLANATION OF FIGURE

FIG. 1. *Dactylella strobilodes*, drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, Prostrate hypha, *a*, from which has been sent up an unbranched erect conidiophore, *b*, that bears terminally a quinqueseptate conidium, *c*; owing to lack of space the conidiophore *b* is shown in three parts whose proper connection is indicated by broken lines. *B*, *C*, Portions of prostrate hyphae, *a*, from each of which

arises an unbranched erect conidiophore, *b*, shown denuded of the single conidium that had been borne on its tip. *D*, Portion of prostrate hypha, *a*, from which has been sent up a conidiophore, *b*, that bears a lateral branch, *c*; the tip of the main hypha, as also that of the branch, is shown denuded of the single conidium that had been borne on it. *E*, Uniseptate conidia, *a* and *b*. *F*, Biseptate conidia, *a–f*. *G*, Triseptate conidia, *a–h*. *H*, Quadriseptate conidia, *a–l*. *I*, Quinqueseptate conidia, *a–j*. *J*, Six-septate conidia, *a–l*. *K*, Seven-septate conidia, *a–p*. *L*, Eight-septate conidia, *a* and *b*. *M*, Nine-septate conidium. *N*, Densely ramifying system of thick lobulate branches found produced on a submerged mycelial hypha.

LEAF BLOTCH OF POPLAR CAUSED BY A NEW SPECIES OF SEPTOTINIA

ALMA M. WATERMAN [1] AND EDITH K. CASH [2]

(WITH 2 FIGURES)

For several years a conspicuous leaf blotch on plantation trees of hybrid poplar clones has been under observation in the eastern United States. The symptoms differ from those resulting from infection by the leaf fungi common on native poplars in that area and the causal organism has only recently been identified as a species of *Septotinia*. This genus was established by Whetzel (**5**) in the subfamily Ciborioideae of the Helotiaceae, based on *S. podophyllina* Whetzel, the only species then known in the genus.

SYMPTOMS

The leaf blotch first appears in early spring as small brown spots on the young developing leaves, frequently at or near the margin, but also at any point on the leaf blade. The spots usually increase rapidly in size, soon become gray at the center, and have an irregular but sharply defined margin. On the most susceptible hybrids large areas of the leaf blade become invaded by the fungus. On the lower surface, the leaf blade and particularly the veins in the affected area become dark brown with white sporodochia along the veins. On the upper surface, small white masses of conidia soon appear, usually developing in concentric circles (FIG. 1, *A*). Along

[1] Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, in cooperation with Yale University, New Haven, Conn., and the Northeastern Forest Experiment Station, Upper Darby, Pa. The earliest observations of the disease were made by Dr. E. J. Schreiner of the Northeastern Forest Experiment Station, who instigated the study of the causal organism. The cultural and pathological phases of the study were carried on by the senior author, assisted by Kenneth F. Aldrich, Biological Science Aid in the same Division.

[2] Associate Mycologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland. The junior author identified the causal organism and prepared the description of the species.

FIG. 1. *Septotinia populiperda.*

the margin of the blotch, white fans of radiating hyphae are frequently formed just below the cuticle of the upper leaf surface, and these, together with a zonate appearance of the entire blotch as the leaf tissue becomes affected, are conspicuous characteristics of the disease. Individual spots may reach a diameter of 1.5 to 2 inches, and two or more of these spots may coalesce, resulting in almost complete invasion of the leaf tissue. Early defoliation may follow and the young stems of the most susceptible hybrid poplars may thus be entirely defoliated by late summer.

IDENTITY OF THE FUNGUS

The fungus present on the leaf blotches produces an abundance of hyaline conidia borne on branched conidiophores which are compactly aggregated to form sporodochia. The conidia are ellipsoid and continuous at first, later becoming 1–3-septate, constricted and frequently breaking apart into two-celled fragments. On examination of the literature it was found that this fungus had been described in 1932 in a paper by Moesz (**3**, p. 41–42, FIG. 5) as occurring on *Populus deltoides* Marsh. in Latvia, under the name of *Septoglocum populiperdum* Moesz & Smarods. A collection on the same host, made in 1930, was also listed by Smarods (**4**, p. 50) without description. Two specimens of *S. populiperdum* on *Populus canadensis* Moench from Latvia deposited in the Mycological Collections of the Bureau of Plant Industry have been examined, and the fungus was found to be identical both in macroscopic and microscopic characters with that found on the poplar hybrids in the United States. No references to *S. populiperdum* other than the two cited have been found. Neither of these articles contains a discussion of the distribution or economic importance of the leaf blotch, but the specific name would indicate the destructiveness of the fungus.

Septoglocum populiperdum differs from typical species of *Septoglocum* in several respects. Its closest affinities are with the conidial stage of *Septotinia podophyllina* which was described as *Glocosporium*, and later referred to *Septoglocum*. Whetzel (**5**) pointed out that the conidial stage of the fungus on *Podophyllum* is not referable to any described genus but felt it unnecessary to

give it a name since the perfect stage is known.[3] The apothecial stage of the poplar fungus, although not yet found in nature, has been developed in culture and found to agree with that of *Septotinia*. Since the spermatial and sclerotial stages characteristic of *Septotinia* have also been found in the fungus on poplar, it is therefore described as the second species of that genus.

Septotinia populiperda Waterman & Cash, sp. nov.

Conidial stage: **Septotis populiperda** (Moesz & Smarods) Waterman & Cash, comb. n. (Syn. *Septogloeum populiperdum* Moesz & Smarods, Magyar Bot. Lapok **31**: 41. 1932).

Sporodochiis amphigenis, saepius epiphyllis, albo-pulverulentis, 80–500 μ in diam.; conidiophoris dense compactis, hyalinis, septatis, 20–24 × 6–12 μ, cellula apicali interdum triangulatim e bullis pluribus ornata, 12–15 μ lata; conidiis hyalinis, unicellularibus vel 1–3-septatis, basibus truncatis, apicibus acutis, ad septa constrictis et dirumpentibus, 15–45 × 4–7 μ; *sclerotiis* per nervos foliorum dispositis, atris, angularibus, elongatis vel circularibus, applanatis, 3–5 × 1–2 mm., interdum coalescentibus; *spermadochiis* e spermatiophoris paucis vel numerosis et dense compactis, verticillatim ramosis, e stirpe centrali oriundis, prope apices compressis dein in forma collaris inflatis compositis; spermatiis globosis, nucleatis, hyalinis, 1.5–2 μ in diam., in catenulis vel massis agglutinatis; *apotheciis* caespitosis, lento-carnosulis, longe stipitatis, breve cupuliformibus, centro plicato depressoque et margine revoluto, hymenio avellaneo, 2–7 mm. in diam., 1–2 mm. altis, stipibus tenuibus, tortis, 1–2 cm. longis, 1 mm. crassis, brunneis; ascis cylindricis, apicibus crasse tunicatis et obtusis, breve pedicellatis, octosporis, 125–135 × 6.6–8.5 μ; ascosporis hyalinis, ovoideis, glabris, aliquantum inaequilateralibus, 10.2–13.2 × 4–5.5 μ; paraphysibus hyalinis, numerosis, flexuosis, ramosis, apicibus ad 1.5 μ inflatis; strato subhymeniali flavidulo, plectenchymatico, exteriori hyalino, pseudoparenchymatico, cellulis 30–40 μ in diam., subglobosis, tenuitunicatis composito.

Sporodochia amphigenous, usually epiphyllous, developing from hyaline subcuticular mycelium on large brownish-gray spots, white, pulverulent, 80–500 μ in diam.; conidiophores densely compact, hyaline, septate, thick-walled, slender below, becoming thicker toward the apex, 20–40 × 6–12 μ, the apical cell sometimes irregularly lobed or triangular, 12–15 μ wide, with 2–3 knoblike protuberances; conidia hyaline, at first unicellular, oblong or acute at one end, 12–15 × 6–9 μ, later 1–3-septate, obtuse at the base, acute at

[3] Buchwald (Contr. Dept. Pl. Path. R. Veter. & Agr. Coll. Copenhagen No. 32, p. 104, 1949) has recently proposed the generic name *Septotis* for the conidial stage of *Septotinia*.

the apex, constricted at the central septum and breaking into 2-celled sections before germination, 2-celled conidia 15–25 × 4–7 μ, 3–4-celled conidia 30–45 × 4–7 μ. *Sclerotia* formed along leaf veins, black, angular, elongate, or circular, flat, 3–5 × 1–2 mm., sometimes coalescing. *Spermadochia* composed of loose to compact groups of verticillately branched hyaline spermatiophores arising from a central stalk, compressed near the apices, then sometimes inflated in the form of a collar; spermatia globose, 1-celled, hyaline, 1.5–2 μ in diameter, each with a large nucleus, often adhering in chains or masses. *Apothecia*[4] single or more often caespitose, tough fleshy, long stipitate, shallow cupshaped, with depressed slightly plicate center and revolute margin, hymenium avellaneous[5] to light cinnamon drab, exterior cinnamon drab to wood brown, 2–7 mm. in diameter, 1–2 mm. deep; stipes long, slender, contorted and spirally twisted, 1–2 cm. long, 1 mm. thick, wood brown becoming bone brown in age; asci cylindrical, wall thickened and somewhat flattened at the apex, slightly narrowed to a short pedicel, 8-spored, 125–135 × 6.6–8.5 μ; ascospores hyaline, ovoid, smooth, unsymmetrical, swollen on one side and depressed on the other, 10.2–13.2 × 4–5 μ; paraphyses numerous, flexuous, hyaline, branched about 20 μ below the apices, 1–1.5 μ thick; subhymenial layer pale yellowish, plectenchymatic, outer layer hyaline, pseudoparenchymatic, composed of subglobose, hyaline, thin-walled cells 30–40 μ in diameter.

On leaves of hybrid clones of *Populus* spp., Maine, Vermont, Connecticut, Massachusetts, New York, Pennsylvania, and Maryland; also on leaves of *P. trichocarpa* Hook. and *P. fremontii* S. Wats., growing in the vicinity of infected hybrid poplars in Maryland.

Apothecia produced in type culture derived from conidia on leaves of clone of hybrid (*P. deltoides virginiana* (Castiglioni) Sudw. × *P. trichocarpa*), FP97101, are deposited in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Md.

Specimens examined: *Septoglocum populiperdum* Moesz & Smarods on *Populus canadensis* Moench. Salaspils, Prov. Vidzeme, Latvia, Sept. 25, 1932, J. Smarods, Fungi latvici exsiccati No. 241; Stopini, Prov. Vidzeme, Latvia, July 20, 1935, coll. J. Smarods, ex Herb. J. Smarods.

[4] Description of apothecia is based on specimens grown in culture.

[5] Color readings are from Ridgway, R., Color standards and color nomenclature. Washington, 1912.

Although very similar to *Septotinia podophyllina* in all of its stages, the species on poplar shows some morphological differences, such as larger apothecia, contorted and spirally twisted stipes, and smaller, unsymmetrical ascospores. The spermatia are also smaller and globose instead of ovate, and the 1- and 2-celled conidia are shorter than those in *S. podophyllina*.

CULTURAL CHARACTERISTICS

Ten monoconidial isolates from leaf blotches on 8 clones of 6 hybrid poplars were cultured for a comparative study of variations among the isolates. Conidia, usually two-celled before germination, produced germ tubes readily from both cells in hanging-drop water culture or on potato-dextrose agar within 18 hours. A vigorous growth of white, fluffy, aerial mycelium developed on both malt extract and potato-dextrose agar. At room temperature colonies developed to about 25 mm. in diameter in a week and spermadochia with abundant spermatia were produced. In monoconidial cultures on potato-dextrose agar in 150 cc. Erlenmeyer flasks, the advancing edge of the colony soon showed a fanlike growth of radiating strands of hyphae similar to that occurring at the margin of a leaf blotch. The aerial hyphae gradually became more appressed to the agar surface, forming a thick mycelial mat. In some monosporous colonies the mycelium remained white, but in others it became tan or yellowish brown, gradually darkening to gray brown. In about 10 days scattered compact masses of mycelium appeared and these gradually darkened, forming black sclerotia (FIG. 1, *B*). In all isolates the sclerotia were circular at first, and in the flask cultures were distributed in concentric circles. The isolates from the various leaf blotches differed considerably in the ultimate size and shape of the sclerotia. Some of these remained circular while others were irregular and later tended to coalesce, forming a thick crust, frequently covered with a grayish-brown mycelial mat. However, the conidia from an individual leaf blotch developed colonies which resembled one another very closely. Sclerotia were abundant in the isolates from some blotches, but were rare in those from others. In the latter, amber-colored gelatinous masses of spermatia were conspicuous on the spermadochia which usually

were arranged in concentric circles (FIG. 2, *A*). This variation in production of sclerotia and spermatia suggests the plus and minus strains reported by Whetzel for *Septotinia podophyllina* (5).

In some of the flask cultures on potato-dextrose agar at room temperature, receptive bodies similar to those described by Drayton for *Sclerotinia gladioli* (2) began to appear about a month after the sclerotia had formed. The receptive bodies (FIG. 2, *B*), which developed singly or in groups directly from the sclerotia, were very slender columns 10–20 mm. long, slightly hairy, cream- or fawn-colored, tapering to a darker apex. The columns became curved or twisted as they lengthened, apparently in response to light stimulus. In agar cultures these bodies failed to develop further and soon withered. Spermatization was attempted by growing two isolates in proximity in a flask and also by pouring over the sclerotia a water suspension of spermatia from another isolate. The receptive bodies, however, showed no response to either treatment. Therefore, a method of spermatization based on that devised by Drayton (2) was developed. Entire sclerotial layers from the potato-dextrose agar cultures with receptive bodies were transferred to flasks containing a three-fourth inch layer of moist sterilized sand. A water suspension of spermatia from another isolate was poured over the sclerotia and also applied to the tips of the receptive bodies by means of a sterilized camel's-hair brush. A thin layer of a moist sterilized mixture of soil and leaf mold was then sprinkled over the surface of the sclerotia. No further development of the receptive bodies took place. In a second attempt at spermatization a water suspension containing a mixture of spermatia from four isolates was similarly applied to sclerotia and receptive bodies in sand cultures, and a thin surface layer of the moist sterilized soil was added. Within a week at room temperature the tips of the receptive bodies began to darken slightly and expand, eventually developing into fawn-colored apothecia (FIG. 2, *B, C*) with mature ascospores. It was found that receptive bodies appeared more rapidly when the sclerotial layer was transferred from potato-dextrose agar to moist sand as soon as it was well developed. By this method the entire process of development from the isolation of a single conidium to the maturing of the ascospores required about 10 to 12 weeks at room temperature.

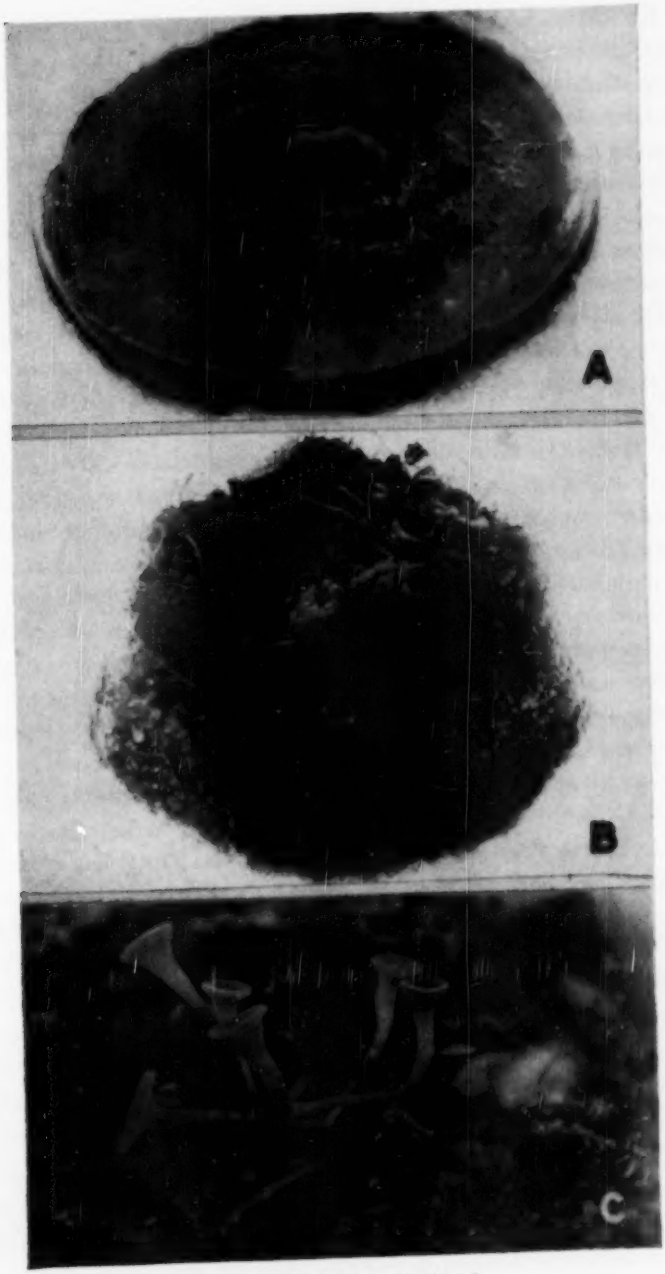

FIG. 2. *Septotinia populiperda.*

Of the ten isolates used in this study only four developed receptive bodies and mature apothecia. In these four, the sclerotia were abundant but spermatia were comparatively rare. The remaining isolates formed an abundance of spermatia, but the sclerotia were mostly limited to the outermost portion of the culture next to the glass of the flask.

No conidia have been produced in culture. The spermadochia, spermatia, apothecia, and ascospores resembled very closely those described and illustrated by Whetzel for *Septotinia podophyllina* (5). However, no study of temperature relations in the growth of the poplar fungus has been made, and color reactions in the agar, reported by Whetzel, have not been observed.

The spermadochia showed considerable variation in development and form. In some cultures they were composed of a short central stalk with a rather loose group of verticillately branched spermatiophores, from the tips of which the spermatia were produced in basipetal succession. The spermatia were held together in gelatinous masses but the general appearance of the mycelial surface of the culture was diffuse and powdery. In other cultures the spermadochia were large compact groups of verticillately branched spermatiophores arising from a stout central stalk which usually was composed of a strand of closely woven hyphae. The spermatia developed in abundance on the tips of the spermatiophores, forming dense gelatinous masses. These masses of spermatia gave an amber-colored waxy appearance to the surface of the culture.

Apothecia have not yet been collected in nature but spermadochia have been found on the blades of fallen leaves and smooth black sclerotia on the veins. Cultures from these sclerotia have produced characteristic mycelial growth, with spermatia and sclerotia.

PATHOGENICITY

The leaves of small potted poplar trees developed from cuttings and growing in the greenhouse were inoculated with a suspension of ascospores. The solution used for the suspension was the sodium-oleate-gelatin mixture devised by Andersen and Henry (1), and made according to the formula which proved to be the most effective spreader-sticker combination in their experiments. Tests with the *Septotinia* ascospores in the mixture in hanging-

drop cultures showed a high percentage of germination in 24 hours.

The species and hybrids of poplars tested were as follows: 2 clones of *Populus fremontii*, 2 clones of *P. trichocarpa*, 2 clones of *P. charkowiensis* Schroed. × *P. trichocarpa*, 1 clone of *P. sargentii* Dode × *P. berolinensis rossica*, 1 clone of *P. petrowskyana* Schneid. × *P. caudina*, 1 clone of *P. candicans* Ait. × *P. berolinensis* Dipp. One tree of each clone, averaging about 14 leaves per tree, was inoculated by placing droplets of the ascospore suspension on both surfaces of each leaf by means of a pipette, allowing the droplets to spread naturally. Satisfactory spread of the suspension was obtained on both leaf surfaces. One additional tree of each of the hybrid clones and one of *P. fremontii* were similarly treated with the sodium-oleate-gelatin mixture without spores and were maintained as controls. All trees were placed under a humidity tent on the greenhouse bench for 48 hours.

Within two weeks small spots appeared on a few of the leaves of the 2 clones of the *charkowiensis* × *trichocarpa* hybrid. The spots developed rapidly and sporodochia with mature conidia were produced. Four weeks after inoculation monoconidial isolations were made on potato-dextrose agar and the resulting cultures developed characteristic spermatia and sclerotia. Leaves of the other hybrid clones failed to develop leaf spots. On the tree of one clone of *P. trichocarpa* a few leaves showed a browning of the midrib and veins suggestive of that occurring on infected leaves under natural conditions but no leaf blotches or sporodochia developed and leaf cultures failed to produce characteristic *Septotinia* mycelium. No indication of infection was evident on the leaves of the trees used as controls.

These limited results, together with field observations, indicate the pathogenicity of the fungus, and the rapid development of the leaf blotch following infection. The conditions favoring the spread of the disease in plantings of hybrid poplar clones and the relative susceptibility or resistance of native and introduced poplar species as well as clones of the hybrid poplars are being studied.

SUMMARY

A leaf blotch on young plantation trees of hybrid poplar clones in the eastern United States is caused by a hitherto unknown spe-

cies of *Septotinia*, described as *S. populiperda*. Large areas of the leaf blades may become affected and extensive premature defoliation may result. Sporodochia with white masses of conidia are found on both surfaces of the leaf spots, usually developing in concentric circles on the upper surface.

Spermadochia with verticillately branched spermatiophores bearing masses of spermatia form on fallen leaves, and small black sclerotia may be found along the veins. Apothecia have not been observed in nature but were produced in pure culture on sterilized sand, following the spermatization of receptive bodies with compatible spermatia.

The leaves of trees of five hybrid poplar clones and of two species of poplar were inoculated in the greenhouse with a suspension of ascospores. Typical leaf blotches with sporodochia and conidia were produced on two clones of the hybrid *Populus charkowiensis* × *P. trichocarpa*.

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EXPLANATION OF FIGURES

FIG. 1. *A*. Upper surface of portion of poplar leaf with blotches, showing white masses of conidia in concentric circles and white fans of radiating hyphae at margins of blotches; natural size. Photograph by R. L. Taylor. *B*. Sclerotia in monoconidial culture on potato-dextrose agar; natural size. Photograph by H. G. Eno.

FIG. 2. *A*. Spermadochia in concentric circles on potato-dextrose agar. *B*. Apothecia developed from cross-spermatization of receptive bodies in flask culture, on sterilized sand, soil, and leaf mold; receptive bodies in the background; natural size. *C*. Mature apothecia in flask culture; three times natural size. Photographs by H. G. Eno.

A NEW GENUS OF THE TREMELLALES FROM LOUISIANA

LINDSAY S. OLIVE

(WITH 2 FIGURES)

During a field trip in a swampy wooded area near Baton Rouge, Louisiana, the writer collected a jelly fungus which seems not to have been previously described. The fungus, which clearly belongs in the Auriculariaceae, was found growing on a fallen dead limb of a frondose tree (FIG. 1, *1*). Although it was found to resemble *Platygloea* in some ways, there are several important features which prevent its inclusion in that genus, at least without a revision of the present concept of *Platygloea*. The writer considers it advisable to erect a new genus to accommodate this new species of jelly fungus.

Mycogloea gen. nov.

Fructificationibus gelatinosis, indeterminatis; basidiis bacillariis et transverse-septatis, sterigmata comparative brevia gerentibus; basidiis gemnatis ex cellis binucleatis persistentibus in modo structuarum vacuarum basibus basidiorum, e quibus facile separabiles sunt.

Type species *M. carnosa*.

Fructifications gelatinous, indeterminate; basidia rod-like, transversely septate, producing relatively short sterigmata, budded out by binucleate cells, which persist as empty structures at their bases and from which they readily become detached.

Mycogloea carnosa sp. nov.

Fructificationibus firmiter gelatinosis, colorem carnosum typice habentibus. Pustulibus, 0.4–2.4 mm. diam., coalescentibus et usque ad 12 mm. longis attinentibus. Hymenio consistente paraphyses, conidiophora, et basidia. Paraphysibus curvatis vel circinatis terminalibus, 1.7–2.5 μ diam., conidiis ovatis vel elongatis, 2.7–3.8 $\times$ 3.8–9.1 μ. Basidiis 3.2–4.9 $\times$ 21–50 μ, angustis, cylindraceis quattuor cellulas habentibus, gemnatis ex binucleatis cellulis subbasidiis, quibus facile separatis sunt, germinantibus et productentibus sporidia vel basidiosporas. Sporidiis 2.4–3.8 $\times$ 3.8–6.1 μ; basidiosporis apiculatis, 2.7–3.4 $\times$ 6.1–8.4 μ. Haec species in frondoso ramo demortuo crescit.

Fructifications firmly gelatinous or cartilaginous-gelatinous, sordid white to light orange, typically flesh-colored, at first appearing as small pustules 0.4–2.4 mm. in diameter, pustules coalescing and forming flattened tuberculate masses up to 12 mm. or more in length, indeterminate. Drying to thin, horny, amber-colored to dingy brown masses. Hymenium containing paraphyses, conidiophores, and basidia. Paraphyses sparingly branched, curved, or circinate at their tips, 1.7–2.5 μ in diameter; conidiophores irregularly branched, producing oval to elongate conidia measuring 2.7–3.8 × 3.8–9.1 μ; basidia straight, narrow, 4-celled, 3.2–4.9 × 21–50 μ, produced by binucleate sub-basidial cells from which they are easily detached at maturity, germinating and producing basidiospores on short sterigmata, or giving rise to an indefinite number of conidia; basidiospores apiculate, flattened on one side, 2.7–3.4 × 6.1–8.4 μ; basidial conidia mostly oval, 2.4–3.8 × 3.8–6.1 μ.

A single collection was made on a dead corticate frondose limb, near Baton Rouge, Louisiana, April 28, 1949.

Microscopic examination of the fungus reveals the presence of a hymenium containing numerous paraphyses, which are characteristically curved or coiled at their tips (FIG. 1, 2). The ends of the paraphyses often undergo gelatinization, a process which can be hastened by the addition of potassium hydroxide solution to the mount. The straight, rod-like basidia arise in proliferating clusters apically and on lateral branches of fertile hyphae with clamp connections, or on lateral branches which originate at different intervals along the lower portions of the paraphyses. The paraphyses extend considerably beyond the basidia. Also present in the hymenium, along with the basidia and paraphyses, are hyphae bearing conidia. These hyphae are irregularly branched, and no clamp connections were found on them (FIG. 2, 5). Conidia are produced in large numbers by these hyphae.

When a fresh mount of the mature fructification is made for microscopic observation, one of the most impressive features is the abundance of detached basidia floating about in the water, along with a large number of conidia (FIG. 1, 2). The basidia apparently become readily detached in nature, many of them rising to the surface of the fructification. It is difficult to find germinating basidia with basidiospores still attached, and most of the basidia seem to bud out indefinite numbers of conidia in a manner characteristic of the basidia of many smut fungi. If basidia are spread out on plain

agar to germinate, the conidia are produced even more profusely (FIG. 2, *1–4*). These conidia are capable of budding in a yeast-like manner. When a mass of basidia is placed in a drop of water on a slide and kept in a moist chamber for a while, most of the basidia produce conidia upon germination. However, many of the basidia

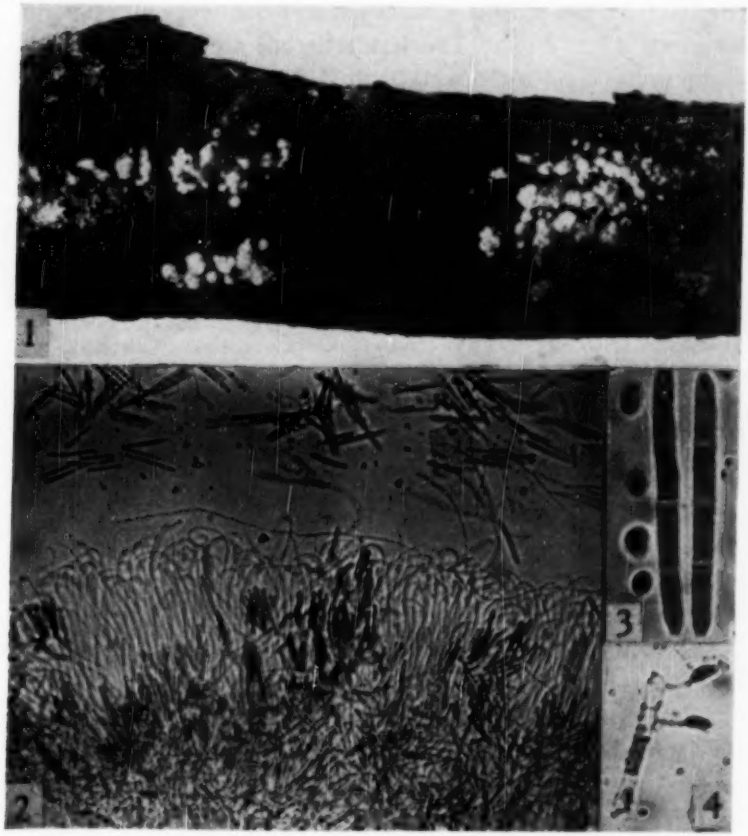

FIG. 1. *Mycogloea carnosa.*

located near the surface of the water produce basidiospores (FIG. 1, *4*; 2, *20, 21*). These spores are produced above the surface of the water. It seems likely, therefore, that in nature the basidia which remain farther down in the fructification produce conidia; whereas, those which drift up closest to the surface after becoming detached

are more likely to produce basidiospores. The latter produce their basidiospores on relatively short sterigmata and resemble the basidia of a typical rust fungus.

With the use of propiono-carmine smear preparations of the hymenium, the writer has been able to make a brief cytological study of the organism. Nuclear details in the conidiophores were not observed, but the conidia were found to be both uninucleate and binucleate (FIG. 2, 6). The fertile hyphae are dikaryotic, with clamp connections at the septa (FIG. 2, 7). The basidia are consistently produced by special binucleate cells which proliferate, with the aid of clamp connections, from the ends of fertile branches. Each basidium is produced in the following manner. A terminal binucleate cell with a clamp connection at its base produces a bud at its tip (FIG. 2, 8). The two nuclei and some cytoplasm migrate into the bud cell (FIG. 2, 9). Both the bud cell and the cell which produces it continue to elongate, and the two nuclei in the bud cell fuse (FIG. 2, 10–12). It is obvious, therefore, that the young bud cell is a probasidium. The cell beneath it is referred to by the writer as a sub-basidial cell. The fusion nucleus now divides and a septum appears across the middle of the basidium dividing it into two uninucleate cells (FIG. 2, 13, 14). A second nuclear division occurs and a 4-celled, 4-nucleate basidium is produced (FIG. 1, 3; 2, 15, 16). These two successive nuclear divisions, in all likelihood, are meiotic. By this time the sub-basidial cell is much elongated, devoid of cytoplasm, and somewhat collapsed (FIG. 2, 17). The mature basidium easily becomes detached from the empty sub-basidial cell.

When the basidium produces basidiospores, each cell gives rise to a sterigma which, in turn, produces a single uninucleate basidiospore at its tip (FIG. 2, 18–21). The basidium is entirely emptied of protoplasm as the four spores are produced. On the other hand, when the basidium produces conidia, several conidia may bud out either directly or on short protrusions from each cell. The basidial nuclei divide and produce the conidial nuclei, and the basidium retains its 4-nucleate condition; the basidial conidia are uninucleate (FIG. 2, 22).

According to Martin (Univ. Iowa Stud. Nat. Hist. **18**: 67.

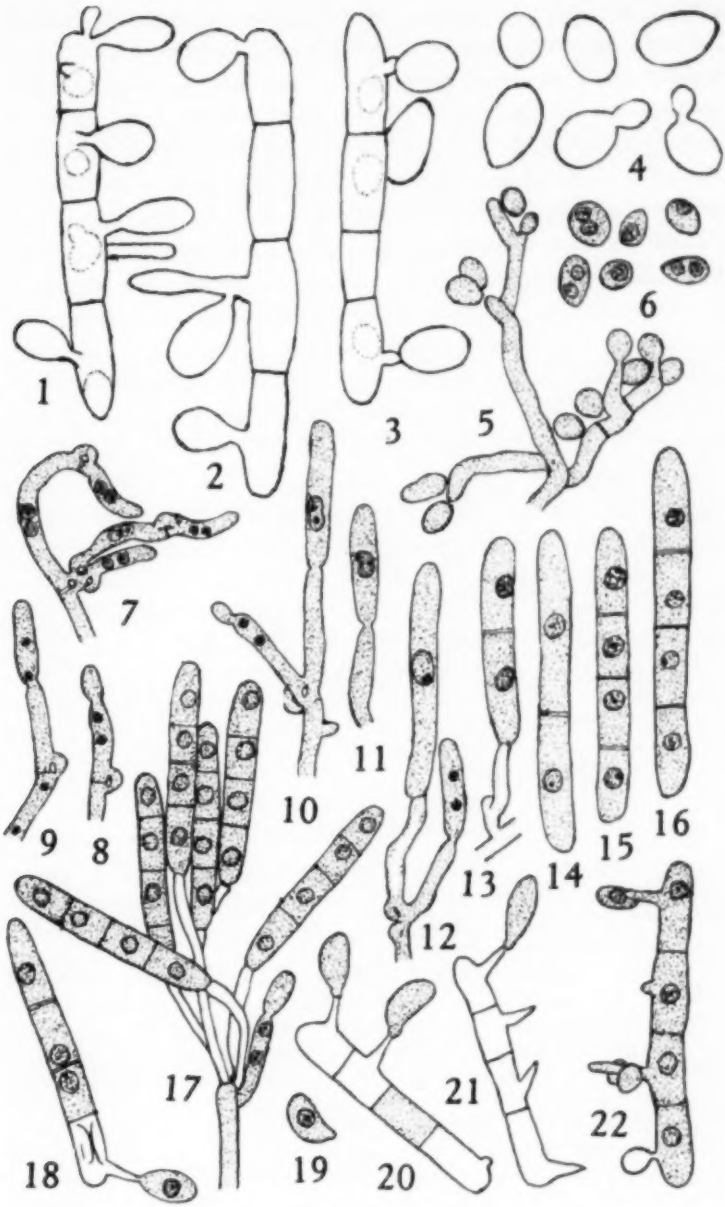

FIG. 2. *Mycogloea carnosa.*

1944), at least one species of *Platygloea*, namely *P. fimicola* Schroet., may have persistent probasidium-like structures ("hypobasidia"). There is no information concerning cytological developments in that species, however. Furthermore, there might be some question as to whether a species with persistent probasidia should be included in *Platygloea*. In any case, the present fungus differs markedly from *P. fimicola* and from all other species of *Platygloea* known to the writer in that it produces straight, rod-like, easily detachable basidia, which may germinate normally to produce indefinite numbers of conidia, or which may produce basidiospores on relatively-short awl-like sterigmata. These are unlike the sterigmata of *Platygloea*, which typically become considerably elongated. The sub-basidial cells are also a conspicuous feature of the new fungus. Although they may superficially resemble persistent probasidial cells at the bases of the mature basidia, they are not considered to be true probasidia, since nuclear fusion does not occur in them, but in the cells budded out by them.

The writer is grateful to Dr. C. W. Edgerton, Department of Botany, Louisiana State University, for his assistance in the preparation of the photographs, and to Dr. Concetta C. Bellini for her preparation of the Latin descriptions.

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EXPLANATION OF FIGURES

FIG. 1. *1–4*. Photomicrographs. *1*, Fructifications on dead wood (× 1.5); *2*, crushed preparation of hymenium, showing curved paraphyses, basidia, and conidia (× 240); *3*, two 4-celled, 4-nucleate basidia and conidia (× 1400); *4*, basidium producing basidiospores (× 780).

FIG. 2. *1–22*. Microscopic study of living and stained material (all figures × 1380). *1–3*, basidia giving rise to conidia on agar; *4*, basidial conidia, some budding; *5*, conidiophores producing conidia; *6*, conidia, uninucleate and binucleate; *7*, hyphae with clamp connections and binucleate cells; *8*, *9*, binucleate cells budding out basidia; *10*, *11*, nuclear fusion in young basidia; *12*, young basidium with fusion nucleus; binucleate probasidium; *13*, *14*, two-celled basidia after first meiotic division; *15*, *16*, mature 4-celled, 4-nucleate basidia (detached) after second meiotic division; *17*, cluster of mature basidia still attached to empty sub-basidial cells; *18–21*, basidia germinating and producing uninucleate basidiospores; *22*, basidium budding out uninucleate conidia.

A NEW ACHLYA FROM MACKINAC ISLAND, MICHIGAN, WITH NOTES ON OTHER SPECIES [1]

T. W. JOHNSON, JR.[2]

(WITH 2 FIGURES)

Several studies relating, at least in part, to the presence of saprolegniaceous fungi in the soils and waters of various localities in Michigan have been published in the past by such workers as Kauffman, Pieters, and Kanouse. The region north of the University of Michigan Biological Station, near Cheboygan, Michigan, has not, however, been the object of any collections made specifically for the Saprolegniaceae, either by these or other investigators. In particular, Mackinac Island has, it would appear, been overlooked as a possible source for these fungi.

Opportunity was afforded on July 18, 1948, of obtaining from the Island, collections of soil and water which might yield saprolegniaceous fungi. The results of this preliminary survey were promising indeed, since, out of a total of 61 collections, 49 isolates were obtained, representing 4 genera, 9 previously described species and one variety, 6 non-fruiting isolates, and one apparently new species. Five of the known isolates, *Achlya Rodriqueziana* F. T. Wolf, *A. flagellata* Coker, *Saprolegnia delica* Coker, *S. monoica* var. *glomerata* ? Tiesenhausen, and *Dictyuchus anomalus* Nagai, have not, heretofore, been reported from Michigan. Figure 1 shows the areas in which collections were made.

Samples were taken from seventeen areas on Mackinac Island. The collections were made from such diverse localities as: water

[1] Contribution No. 898 from the Department of Botany, University of Michigan.

[2] The author wishes to express his deepest appreciation to Professor F. K. Sparrow, for his helpful suggestions and criticisms in the preparation of this paper, and to Mrs. T. W. Johnson, for assistance in preparation of the figures.

FIG. 1. Sketch of Mackinac Island, Michigan, showing areas of collection and points of orientation. Scale: 1" = appx. 800 yards.

from beach pools and inland pools; sandy, dry soil from the margins of beach pools and near the vegetation line; humus from the forest floor; muck from an intermittent bog, and soils and water from marsh areas adjacent to the beach line. An abundance of dead, young lake trout (*Cristovomer* sp.) bearing a cottony mycelial growth were found in a few of the beach pools. Three of these fish were taken as part of the collections.

The samples were subjected to the usual well-known methods of obtaining these fungi. Single spore isolates were made of all species as they were recovered from the samples. All identifications and descriptions are based on colonies 12–16 days old, growing on half of a boiled hempseed, in 30 cc. of sterile, charcoal-filtered, distilled water, at a temperature of $22 \pm .4°$ C. Percentages, averages, *etc.*, are reported on the basis of 500–700 observations or measurements of the morphological features concerned.

Three collections from the Island yielded a fungus, intermediate between *Achlya Klebsiana* Pieters and *A. Rodriqueziana* F. T. Wolf. The plant, however, is sufficiently distinct from these and other known species to be considered as new.

Achlya michiganensis sp. nov.[3] FIG. 2

Myceliis in semine *Cannabis sativae* densis, quandoque porrectis usque ad 1–1.5 cm. in diametrum; hyphis primariis in basi 40–50 μ diam. Sporangiis copiosis, cylindratis aut attenuatis, inter 152–210 μ longis et 17–22 μ diam.; cymosis, zoosporiis 9.9–11.0 μ diam., germinantibus sicut in Achlyis; aliquibus secondariis sporangiis excrescentibus ex 1–3 lateralibus papillis. Gemmis paucis, variis, plerumque inaequaliter formatis. Oogoniis copiosis et globosis; natis ex primariis hyphis in ramulis lateralibus, curvis aut raro rectis, nonnumquam semel usque ad quater aut magis pro diametro cuiusque oogonii, sed plerumque bis usque ad ter; raro terminalibus, numquam intercalaribus; 35–66 μ diam., plerumque 38.5–49.5 μ; proliferentibus et prolem crescentibus abunddanter; tunica levi et tenui, notata nullis punctulis nisi ubi antheridia tangunt. Oosporiis globosis aut ellipticis; excentricis; inter 18.7–28.6 μ diam., sed plerumque inter 22.0–25.3 μ; numero 1–6, plerumque 3, rarissime 8–10. Antheridiis frequentissime dicliniis nonnumquam irregularibus et contortis; persistentibus; antheridio digitalibus prominentiis affixo, 1–4 pro quoque oogonio.

Hab. ad terram humosam, Mackinac Island, Michigan, July 18, 1948.

[3] Grateful acknowledgment for the preparation of the Latin diagnosis is extended to Professor Bruno Meinecke, Department of Classical Studies, University of Michigan.

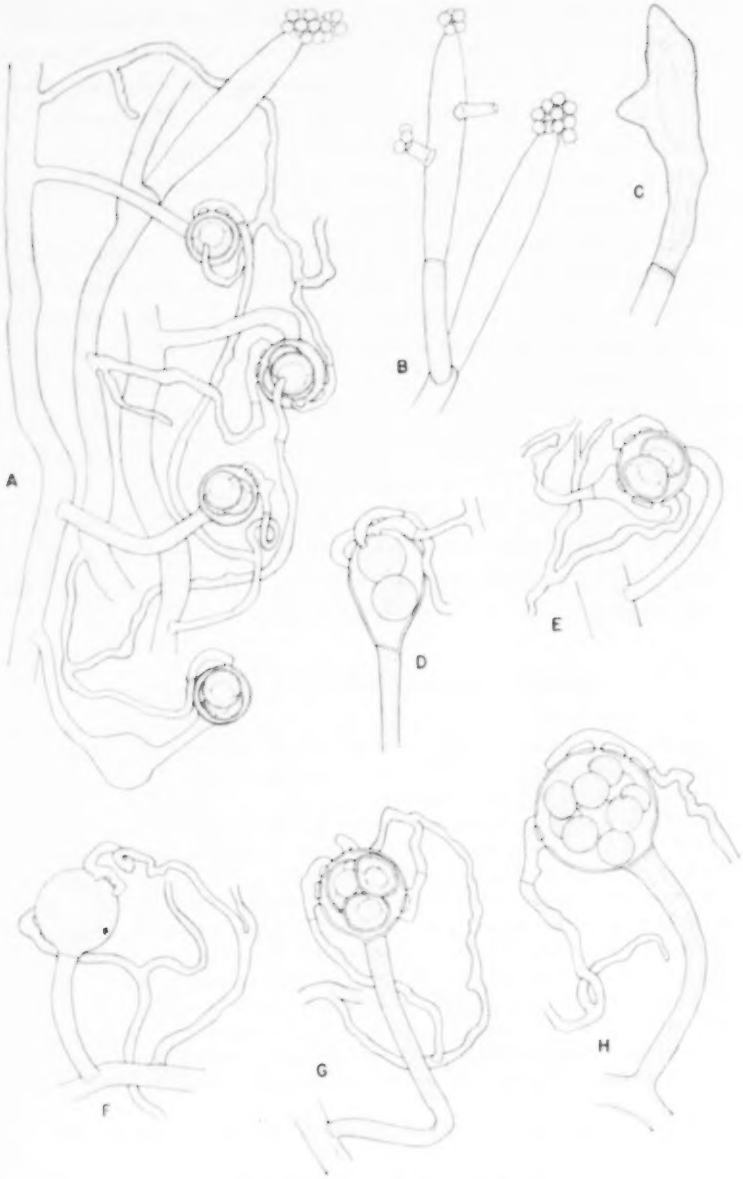

FIG. 2. *Achlya michiganensis.*

Growth on hempseed dense, the colony averaging 1–1.5 cm. in diameter; principal hyphae 40–50 μ in diameter at the base. Gemmae scarce, even in very old cultures; variable in size; irregular; mostly terminal and single, or not in chains of more than two. Sporangia abundant; cylindrical, or tapering toward the apex; predominately broadest near the middle; 112–280 × 14–28 μ, usually 152–210 × 17–22 μ; renewed only by cymose branching; primary zoospores escaping through an apical pore and encysting to form a hollow sphere; in occasional secondary sporangia also escaping through 1–3 long, lateral exit tubes; encysted spores 9.9–11.0 μ in diameter. Oogonia abundant, spherical or short-pyriform; borne laterally along the main hyphae on bent or rarely straight stalks 1–4 or more times longer than the diameter of the oogonium, usually on stalks 2–3 times the diameter of the oogonium; rarely terminal, never intercalary; 35–66 μ in diameter, predominately 38.5–49.5 μ; proliferation of unfertilized oogonia common; wall smooth, thin, unpitted except where antheridial cells are attached. Oospores spherical or ellipsoidal; eccentric, with a single oil droplet not completely surrounded by protoplasm; 18.7–28.6 μ in diameter, predominately 22.0–25.3 μ, averaging 23.7 μ in diameter; in large, abnormal oogonia, predominately 19.0–20.1 μ; 1–6, usually 3 in number, very rarely 8–10[a]; germination not observed. Antheridial branches predominately diclinous; if androgynous, the branch usually originating remote from the oogonium and its stalk; long, much-branched, irregular and contorted; persistent; antheridial cells clearly abstricted, and attached to the oogonium by finger-like projections; 1–4 antheridia to an oogonium; fertilization tubes rarely formed.

From wet humus and sandy soil, Catholic Cemetery, Area 12 (**Type**); dry, sandy soil, near Military Cemetery, Area 13; wet debris and sand from below water line in a pool, Old Fort Garden, Area 16. Mackinac Island, Michigan, July 18, 1948.

The small number of oospores, the small, smooth-walled oogonia which tend to proliferate when unfertilized, the bent or curved oogonial stalks, and the much-branched, irregular antheridial branches, constitute the distinctive features of this fungus.

Achlya michiganensis has been compared with living material of *Achlya Klebsiana*, *A. Rodriguezziana*, *A. americana*, *A. flagellata*, and several of the closely related papillate species of the genus.

[a] About 5% of the oogonia with 1 oospore, 25% with 2, 40% with 3, 15% with 4, 10% with 5, 5% with 6, and less than 2% with 8 or 10.

The affinity of this Michigan plant to *A. Klebsiana* and *A. Rodriguezziana* is at once obvious.

Achlya michiganensis differs from *A. Klebsiana* as defined by Pieters (**8**) in the following features: possession of pits in the oogonial walls at the point of attachment of the antheridial cells; formation of longer, bent or recurved oogonial stalks; presence of an occasional androgynous antheridium; frequent proliferation of unfertilized oogonia, and a lesser number of smaller oospores. The living material of *A. Klebsiana* (kindly furnished by Professor J. N. Couch) has characteristics not in complete agreement with the fungus as described by Pieters. This material has, nevertheless, been used as a basis for comparison, although with the reservation that it may not be *A. Klebsiana*. In the final analysis, Pieters' description of *A. Klebsiana* must necessarily be accepted as the correct interpretation. The sporangia which discharge the spores only by an apical pore; the occasional presence of 1–3 long, lateral exit tubes in some secondary sporangia; scarcity of gemmae; more pronounced curve or bend to the longer oogonial stalks; smaller size of the oogonia; the lesser average number of oospores; the much-branched, irregular antheridial branches, and the frequent proliferation of unfertilized oogonia in *A. michiganensis*, are points of distinction between it and the material supplied by Professor Couch.

With respect to oogonial size, eccentricity of oospores, and proliferation of oogonia, there is a resemblance between *A. michiganensis* and *A. Rodriguezziana* (**11**). On the other hand, the unpitted oogonial walls, smaller number of oospores, fewer androgynous antheridia, parthenogenetic maturation of some unfertilized oogonia, more abundant gemmae, and more extensive growth of *A. Rodriguezziana*, serve to distinguish it from the Mackinac Island isolate.

Proliferation of oogonia in *Achlya proliferoides* and *A. flagellata* is suggestive of *A. michiganensis*, but beyond this similarity, the two known species are obviously distinct from the present fungus. The illustrations of *Achlya Oryzae* Nagai (**7**), from Japan, are suggestive of *A. michiganensis*. Nagai's species may readily be separated from our plant on the basis of the possession of larger

oogonia, pitted oogonial walls (rarely not pitted), larger number of oospores, ramified or clasping antheridial cells, and the more frequent androgynous antheridia. Our species is also decidedly different from any of the European species of *Achlya* thus far described.

Preliminary studies concerning the effect of natural environmental conditions (clear stream water, stagnant pool water, *etc.*) on the distinguishing morphological features of *A. michiganensis*, would seem to indicate that the fungus is consistently stable in its characteristics. The formation of more abundant gemmae was not induced by any of the cultural conditions which were utilized. The effect of low temperatures on the production of papillations on the oogonial walls of *A. michiganensis* was also studied. Temperatures of 10, 15, and 20° C. were ineffective in inducing papillate oogonia.

OTHER FUNGI RECOVERED

Saprolegnia parasitica Coker. This species was obtained from the following localities: dead lake trout, in beach pool south of Grand Hotel, Area 1; beach pool near Pontiac's Lookout, Area 5, and beach pool below Lover's Leap, Area 6. Attempts to induce sex organ formation by cultivation of the isolates on the glucose-peptone medium suggested by Kanouse (**5**), were successful in only one of our isolates. In these colonies the fructifications were comparable to those described by Kanouse for *S. parasitica*. The vegetative and asexual features of the sterile isolates were characteristic of the species.

Saprolegnia ferax (Gruith.) Thuret. Isolated from wet humus and sandy soil taken from the bank of Brown Brook, Area 9. Irregular sporangia; large, pitted oogonia which have a tendency toward clustering, and rarely-formed antheridia characterize this species. Another morphological feature which appears of some value in the identification of *S. ferax* is the formation of cylindrical oogonia in old sporangia. In the first of our isolates, Strain M9-1, this feature is of common occurrence, while in the second isolate, Strain M9-2, it is quite infrequent. The percentage of antheridia has varied considerably in our two strains from culture to culture. Strain M9-1 produced antheridia varying from 1.5–76% in twelve

consecutive cultures. Strain M9-2, on the other hand, produced antheridia on .6–11% of the oogonia in the same number of subcultures, grown under similar conditions. The oospores in Strain M9-1 were slightly smaller than those reported for *S. ferax*, while in Strain M9-2 these structures agreed in size with the species as interpreted by Coker and Matthews (2).

Saprolegnia mixta deBary. Collected from sandy soil below Stonecliff, Area 7; water from beach pool 200 yards northeast of Stonecliff, Area 8; soil and humus near cemeteries, Areas 11 and 12, and from water and debris, east edge of Marsh, Area 15. This ubiquitous species has been identified with difficulty. Only the isolate recovered from Area 15 was truly comparable to the species as understood by Coker and Matthews. The other isolates have varied from the type, particularly with regard to the number of oospores, and the size of oogonia. The percentage of antheridia formed in our collections has been so variable, ranging from 15–90%, that it was of little value in the determination of the species of the majority of our isolates. The percentage of antheridia for *S. mixta*, as described by Coker and Matthews, is "40% or more but not on all of the oogonia." None of our isolates has produced cylindrical oogonia in old, discharged sporangia, and it is primarily on this basis that our strains have been separated from *S. ferax*, a closely-allied species.

Saprolegnia delica Coker. Collected from soil and debris near vegetation line at bathing beach, Area 4, and from soil and debris near vegetation line, Pontiac's Lookout, Area 5. Our collections differ from the species as defined by Coker (1) only in the fewer number of small, abnormal oospores formed in the large oogonia. Up to 40 such oospores were reported by Coker; only up to 28 have been observed in the oogonia of our isolates.

Saprolegnia diclina Humphrey. Recovered from soil and humus, Protestant Cemetery, Area 11, and from Military Cemetery, Area 13. The occasional occurrence of oogonia in chains is reported in *S. diclina* by Coker (1), although Humphrey (4) makes no mention of this feature. In the Mackinac Island material of this species, grown under the conditions previously outlined, the appearance of oogonia in a moniliform arrangement is rather infrequent.

The percentage of such organs has been altered from 2–55 by varying the temperature at which the cultures were grown. The highest percentage of moniliform oogonia was obtained in a culture developing at 10 ± .3° C., while the lowest percentage was found in a culture grown at 22 ± .4° C. With respect to the other features which identify *S. diclina*, our isolates agree remarkably well, particularly in the disappearance of the antheridial branches soon after the antheridial cells are abstricted.

Saprolegnia monoica var. *glomerata* (?) Tiesenhausen. This variety was found in water and debris taken from a beach pool at the southeastern end of the bathing beach, Area 3. Our collection resembles quite closely Coker's conception of the variety, but is markedly different from Tiesenhausen's original description (**10**). Three points of distinction between our isolates and Tiesenhausen's may be made. First, the oospore number in the variety *glomerata* is less than in our material. Second, the contortions, clustering, or irregularities of antheridial branches, characteristic of the variety, are not found in the Mackinac Island material. Third, the terminal oogonia of the variety *glomerata* are reported to be usually cylindric in old sporangia, a feature never observed in the isolates which we have collected.

Three non-fruiting members of the genus *Saprolegnia* were collected from beach pool water, west of Grand Hotel, Area 2; from water, west edge of marsh, Area 14, and from soil, margin of Old Fort Garden pool, Area 16.

Achlya americana Humphrey. Recovered from water and debris, bathing beach, Area 4, and from humus in intermittent bog, Area 10.

Achlya flagellata Coker. Collected from soil below Stonecliff, Area 7.

Achlya Rodriguezziana F. T. Wolf. Isolated from soil and debris in Old Fort Garden, Area 16. Our material of this plant resembles the characteristics of the type with the exception of slight variations in the percentages of oospore number, when compared with the percentage of frequency of these structures as reported by Wolf (**11**). This species has also been collected in Illinois (Shanor, personal communication). The Illinois material has

characteristics, however, which are not in complete agreement with the type of the species.

Two sterile isolates of *Achlya* were recovered from two collections of water in Area 8, 200 yards northeast of Stonecliff.

Dictyuchus anomalus Nagai. Isolated from soil and humus, intermittent bog, Area 10. This interesting plant has previously been reported from Florida (3), and from North Carolina (Couch, personal communication). It has appeared in several of the writer's collections from the Lower Peninsula of Michigan. The fungus is quite easily recognized by the complete lack of antheridia. Our strain differs from the strains collected by Couch, only in the prolific production of oogonia.

Dictyuchus sp.? Presumably heterothallic strains collected from humus and soil, west edge of marsh, Area 14, and from soil, Scott's Cave, Area 17. No sexual reproductive structures have been induced by any of several cultural conditions to which the plants have been subjected. Sporangia are all of the true-net type. With regard to the sporangial morphology and vegetative characteristics of the isolates, there is an obvious resemblance to *D. monosporus* Leitgeb (*D. sterile* Coker). Several attempts have been made at crossing the Mackinac Island material with other supposedly heterothallic strains of *Dictyuchus*, collected elsewhere in Michigan, but these have met without success. It is assumed, then, that we have only non-complementary strains, and until our isolates can be paired with compatible strains with the resulting production of sex organs, positive identification cannot be made.

Isoachlya, non-fruiting. Isolated from soil near Scott's Cave, Area 17. As in the previous non-fruiting isolates herein listed, vegetative and asexual characters have necessarily been used in the identification of this plant. Considering these features, there is no doubt that our isolate is a member of the genus *Isoachlya*. We have made several attempts to induce sex organs by means of variations of temperature and nutrition, but these have not been successful.

A fungus was recovered which closely resembles *Saprolegnia monoica* Pringsheim, previously reported from Michigan by Pieters

(**6**, **9**). Because of incompleteness of description of *S. monoica*, an extensive taxonomic study of our fungus is being conducted, the results of which are to be reported at a later date.

SUMMARY

The results of a preliminary survey of the Saprolegniaceae in the soil and waters of Mackinac Island, Michigan, are reported.

The fungi recovered are distributed in four genera, and consist of nine previously described species and one variety, and six non-fruiting isolates. A new species of *Achlya*, recovered from the soil of three areas on the island, is described as *Achlya michiganensis*.

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EXPLANATION OF FIGURE 2

Achlya michiganensis

FIG. 2. A. Habit sketch of portion of mycelium, showing the distinguishing features of the fungus when grown on hempseed. B. Primary and secondary sporangia, showing method of spore discharge, and short cymose branching. Secondary sporangium with two lateral discharge tubes. C. Typical gemma. D. Terminal oogonium with immature oospores, and diclinous antheridia, one of which is abnormally short. E. Lateral oogonium with two mature oospores, showing a strongly curved oogonial stalk, and branched, irregular, diclinous antheridia. F. Immature oogonium with both a diclinous and an androgynous antheridium attached. G. Oogonium with three mature oospores, showing a very long, bent oogonial stalk, and a branched, diclinous antheridium. H. Large, abnormal oogonium with ten small, immature oospores, and two diclinous antheridia. All drawings made with aid of Spencer camera lucida. Fig. A, appx. $\times 620$; fig. B, $\times 410$; all others, $\times 840$.

RAMULARIA LEAF SPOTS OF LATHYRUS ODORATUS AND L. LATIFOLIUS

KENNETH F. BAKER, WILLIAM C. SNYDER, AND LILY H. DAVIS

(WITH 2 FIGURES)

In June, 1933, a *Ramularia* leaf spot of sweet pea (*Lathyrus odoratus* L.), and in July, 1938, a similar leaf spot of perennial pea (*L. latifolius* L.), were first collected in California in Santa Barbara County.[1] We have subsequently observed these diseases on many occasions in coastal areas of the state.

The leaf spots resemble closely those of *Lathyrus* spp. attributed in the literature to species of *Scolicotrichum, Ovularia, Ramularia, Cladosporium, Hyalodendron,* and *Erostrotheca.* The question arose, therefore, as to the possible identity of the California fungi with those described elsewhere, and the proper binomial of the pathogen. The conclusions reported here are based on observations of field material, cultures of the fungi, inoculation tests, and examination of the exsiccati cited here, during a period of several years.

SYMPTOMS

On sweet pea the symptoms are the same as those described and illustrated by various workers (**14, 19, 37**). On leaves the buff-colored dead areas are circular or irregular and merge gradually into healthy tissue. Sporulation occurs as white tufts visible with a hand lens on both sides of the leaf but most abundantly on the undersurface. The spots vary from small yellow flecks to areas involving most of the leaf, and leaf fall is usually hastened. Lesions may occur on the wings of stems.

On perennial pea the spots occur on leaflets, petioles, stipules, tendrils, stems (FIG. 1, C), peduncles, and pods. They are typi-

[1] Deposited in the Herbarium, Division of Plant Pathology, University of California, Berkeley, under the accession numbers P 568 (collected by L. M. Massey and M. W. Gardner) and P 695 (collected by W. C. Snyder), respectively.

cally tan to dark brown with definite margins usually darker in color than the center. Commonly the leaf spots are restricted laterally by large veins and tend to be elongated (FIG. 1, A, D). The basal parts of plants in seed fields are frequently severely spotted, probably because of the high humidity under the dense plant growth. The conidial tufts on this host appear on both leaf surfaces, but are most abundant on the underside (FIG. 1, D, E); they are distinctly pink in color.

The disease on sweet pea occurs but is not important in outdoor and clothhouse cut-flower plantings in coastal California. It is less common in seed fields because of the drier conditions of growth.

The disease on perennial pea is much more common and conspicuous in seed fields because of the perennial nature of the plant, the more vigorous growth, and the practice of ditch irrigation. It is, however, unimportant on this minor crop. Lesions on the pods might extend through the pod walls, infecting the seeds, but such seed transmission was not investigated.

There are few diseases of sweet pea or perennial pea in this country that might be confused with *Ramularia* leaf spot. Powdery mildew (*Erysiphe polygoni* DC.) occurs commonly over the plant and forms sparse to dense, superficial, white mycelial growth without fasciculate tufts, and sometimes involves all of the leaf and stem surfaces. The *Ramularia* leaf spots are definitely localized and have dead centers in which small white to pink tufts of conidiophores are evident, without superficial mycelial development.

Anthracnose [*Glomerella cingulata* (Ston.) Spauld. & Schrenk] is said to cause white or pallid leaf spots and to girdle stems, producing dead tips. Acervuli bearing slimy salmon-colored masses of spores occur in these spots. The *Ramularia* disease is not known to kill shoots, and produces dry, pink or white, conidial tufts.

Ascochyta blight [*Ascochyta* spp., *Mycosphaerella pinodes* (Berk. & Blox.) Stone] is reported to produce dark brown to purple lesions on the stems, foliage, and pods, or light brown indefinite spots on the leaves, apparently depending upon the species of *Ascochyta* involved. In all cases, however, light to dark brown pycnidia form in the blighted tissues followed later, in some in-

stances, by perithecia of the *Mycosphaerella* state. The presence of pycnidia which exude their conidia in tendrils, and the lack of aerial development of the fungus on the surface readily distinguish this disease from *Ramularia* leaf spot.

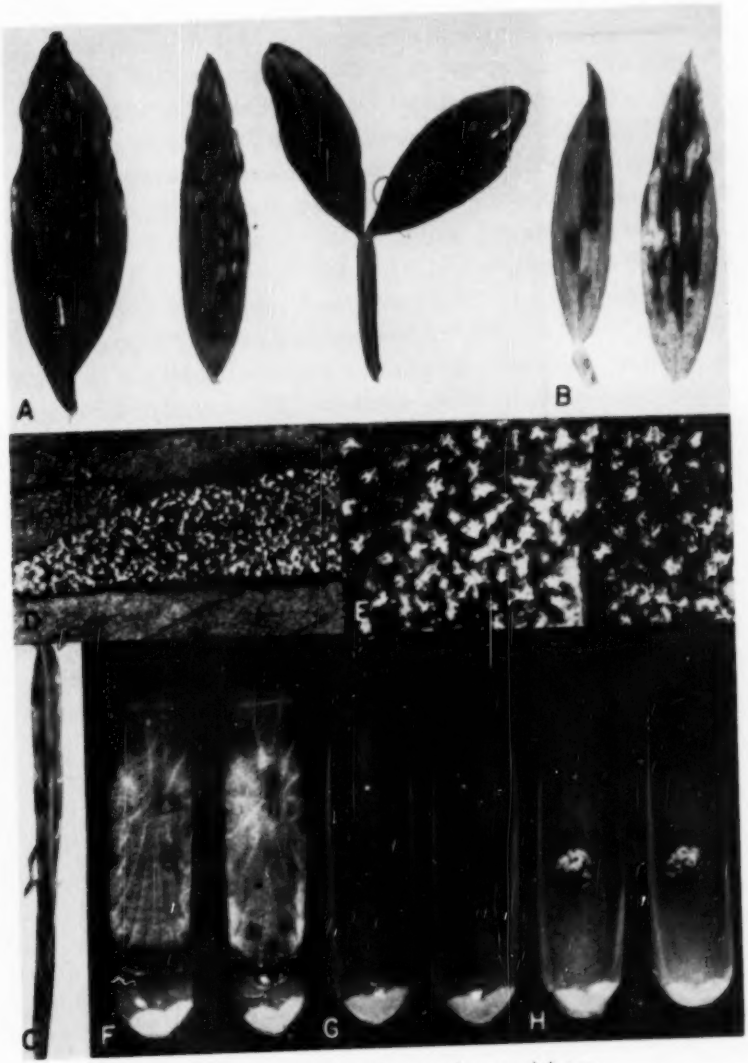

FIG. 1. *Ramularia* leaf spot of perennial pea.

The common name that seems most appropriate for the *Ramularia* diseases is *Ramularia* leaf spot. The disease of sweet pea has been called white blight, white mold, and *Cladosporium* blight.

GEOGRAPHICAL DISTRIBUTION

Pathological studies with the organism were first conducted by Dowson (**14**) in England in 1922 on sweet pea, but Lind previously had collected it on that host in Denmark in 1913 (Kabát et Bubák, Fungi imperfecti exsiccati No. 791). The disease on this crop was first reported in the United States at Reading, Massachusetts on April 27, 1927 (collection of E. F. Guba), and may have been present by 1922 (**3**). It was found in the same year in New York, and in Pennsylvania in 1928 (**37**), in New Jersey from 1932 to 1934 (**49**), in California in 1933 and in Texas in 1942 (**1**). It was reported to the Plant Disease Survey by C. E. F. Guterman as *Ramularia* sp. on sweet pea on Long Island, New York in 1933; specimens collected there by him in 1935 were referred to *Cladosporium album*. In Canada it was first seen in 1931 in British Columbia (**23**), and it was again reported there in 1933, 1944, and 1947; it was found in Ontario in 1945 (**19**).

An *Ovularia* sp. on perennial pea (*L. latifolius*) was collected in California by G. L. Grisvold and A. D. Cameron in 1943, according to Plant Disease Survey records. *Cladosporium album* was reported on this same host in Washington in 1943 (U. S. Dept. Agr., Mycol. Dis. Surv. herbarium, Forsell and Ramsey, October 29, 1943).

There are many mycological records of what appears to be the same fungus on various *Lathyrus* spp. over much of the temperate and subtropical world. Distribution records given in this paper are summarized below with a reference to the probable earliest collection in each country: Germany in 1869 (**17**; also Fuckel, Fungi rhenani exsiccati No. 2206); Siberia in 1877 (**48**); Belgium in 1887 (**6**); France in 1898–1899 by Fautrey (**28**); Italy in 1901 (**16**); Hungary in 1910 (**24**); Denmark by 1913 (**33**); Sweden in 1914 (**9**); Czechoslovakia in 1916 (Petrak, Flora Bohemiae et Moraviae No. 1253); Holland in 1920 (**41**); England in 1922

(**14**); Caucasus in 1923 (**47**); Alaska in 1923 (Anderson, Alaska Fungi No. 994); Estonia in 1925 (**32**); United States in 1927 (**3**); Lithuania in 1927–1932 (**8**); Poland in 1930 (**18**, p. 203); Canada (British Columbia) in 1931 (**23**); Ireland in 1933 (**39**); Latvia in 1934 (K. Starcs Herbarium No. 2337); and Scotland in 1942 (**12**). The evidence suggests that the fungus may be native to Europe and Asia.

HOST RANGE

The host plants are given below with a reference to the first apparent record on each: *Lathyrus tuberosus* L. (**17**); *L. pratensis* L. (**48**); *L. sylvestris* L. (Fautrey, *in* **28**); *L. hirsutus* L. (**16**); *L. odoratus* L. (Kabát et Bubák, Fungi imperfecti exsiccati No. 791); *L. maritimus* (L.) Bigel. (**9**); *L. roseus* Stev. (**47**); *L. palustris* L. (Anderson, Alaska Fungi No. 994); and *L. latifolius* L. (Sydow, Mycotheca Germanica No. 2435).

Plants outside of the genus *Lathyrus* have been reported as hosts of this fungus. Among these are: *Lotus* (Tetragonolobus) *siliquosus* L. (**34**); *Vicia* sp. ("Chickling vetch") (**15**); *Vicia* sp. (**40**); *Pisum* sp., *Phaseolus* sp., and *Vigna* sp. (**21, 22**).

Good (**19**) was unable to establish infection with the sweet pea fungus on *Lathyrus latifolius*, *Vicia faba* L., *V. villosa* Roth., *Melilotus alba* Desr., *Medicago sativa* L., *Pisum sativum* L., *Phaseolus vulgaris* L., *Trifolium pratense* L., or *Glycine max* Merr. Dowson (**14**) was unable to infect *Pisum sativum* and *L. aphaca* L. These unsuccessful inoculations may be explained on the basis of the existence of physiologic races (**19**).

NOMENCLATURE OF THE FUNGUS

There are a number of possibilities to be considered in the selection of the most appropriate binomial for this fungus. These are discussed here chronologically.

1. *Ovularia fallax* (Bon.) Sacc. This fungus, described by Bonorden (**7**) as *Crocysporium fallax* Bon. on *Vicia* sp., was placed by Saccardo (**43**, vol. 13: 1307) in Ovularia. It is now known (**35, 44**) from examination of specimens that this was the conidial stage of a powdery mildew, and is, therefore, invalid.

2. *Ovularia deusta* (Fuckel) Sacc. This fungus was described by Fuckel (**17**) as *Scolicotrichum deustum* Fuckel, causing deep brown to black spots on living leaves of *Lathyrus* (Orobus) *tuberosus* in the fall at Eberbach, Germany. Saccardo (**43**, vol. **4**: 140–141) placed this under *Ovularia deusta* and repeated Fuckel's description practically verbatim. The specific name *deusta* (from the Latin *deustus*, burnt) may have been used because of the dark appearance of the spots or perhaps because of the tiny, pink, conidial clusters conspicuous in Fuckel's specimen. The spores were $4 \times 12\,\mu$, lanceolate, continuous, and hyaline; conidiophores were fasciculate, pink, simple, slender, and bore terminal conidia.

The binomial *O. deusta* has been subsequently used by many workers down to the present day. Among these are: Bommer and Rousseau (**6**) in Belgium in 1887; Kirchner (**30**, pp. 143 and 439) in Germany in 1890; Knet (Fungi Schemnitzienses, collected in Germany by Sitno, August 20, 1897); Lindau (**34**) in Germany; Lind (**33**, p. 499) in Denmark; Briosi in Italy in 1912 (**2**); Marchal (**36**) in Belgium between 1913–1919; Petrak (Flora Bohemiae et Moraviae No. 1253) in Czechoslovakia in 1916; Jaap (**27**; also Fungi selecti exsiccati No. 844) in Germany in 1916; Siemaszko (**47**) in the Caucasus in 1923; Sydow (Mycotheca Germanica No. 2224 [FIG. 2, I] and No. 2435) in Germany in 1923 and 1924; Brundza (**8**) in Lithuania between 1927 and 1932; Hruby (**25**) in Czechoslovakia in 1928; Garbowski and Juraszkówna (**18**, p. 203) in Poland in 1930; Eriksson (**15**, p. 444); O'Connor (**39**) in Ireland in 1933; Muskett *et al.* (**38**) in Ireland in 1934; and Chesters (**10**) in England in 1937.

Examination of Fuckel's Fungi rhenani exsiccati No. 2206 in the Farlow Herbarium[2] showed this organism (FIG. 2, H) to be the same as the one that occurs in California. The legend on this specimen is as follows: "2206. *Scolicotrichum deustum.** Ad Orobi tuberosi folia marcescentia frequens. Autumno. *Von allen, hier neu aufgestellten, aber nicht beschriebenen, Arten und Gattungen befinden sich die ausführlichen Beschriebung in meinen im Drucke befindlichen 'Symboliae mycologicae.'"

3. *Ramularia Galegae* Sacc. f. *Lathyri* Ferraris. The species

[2] Examined through the courtesy of Dr. W. L. White.

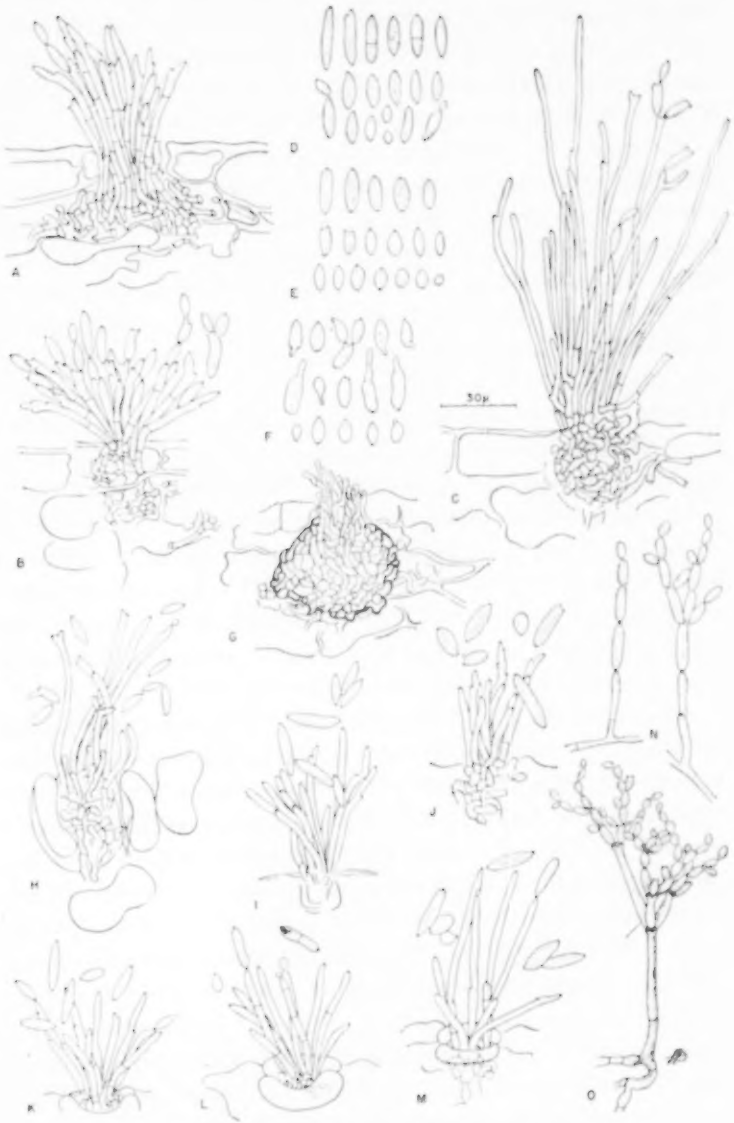

FIG. 2. *Ramularia* leaf spot of perennial pea.

was described by Saccardo (**42**) on *Galega officinalis* L. in Italy. The form was reported on *Lathyrus hirsutus* L. in Italy in 1901 (**16**) producing spots which lacked definite margins. The conidiophores were fasciculate and hyaline; the conidia were continuous or rarely 2-celled, hyaline, 16–26 × 3–3.5 μ.

The fungus was said to have been collected by Fautrey on *L. sylvestris* in France in 1898 and exsiccati were distributed as Herbier Cryptogamique de Côte d'Or No. 2630. Ferraris later changed his original binomial to *R. Lathyri* Ferr. (**28**). The situation was reviewed by Keissler (**28**) and the conclusion reached that *R. Lathyri* Ferr., *R. Lathyri* (Fautr.) Ferr., *R. Galegae* f. *Lathyri* Fautr., and *R. Galegae* f. *Lathyri* Fautr. apud Ferr. are all synonymous.

Bubák (**9**) also considered *R. Galegae* Sacc. var. *Lathyri* Ferr. and *R. Lathyri* Hollós, both on *Lathyrus hirsutus*, as synonymous and changed the former to *R. Lathyri* (Ferr.) Bubák.

Lind collected *R. Galegae* Sacc. f. *Lathyri* Ferr. on *Lathyrus odoratus* in Lyngby, Denmark on September 28, 1913 (Kabát et Bubák: Fungi imperfecti exsiccati No. 791). This appears to be the first record of the fungus on cultivated sweet pea. Stares found the fungus in 1934 in Latvia on *L. odoratus* and called it *R. lathyri* Ferr. (K. Stares Herbarium No. 2337).

All of these names are later than *Ozularia deusta*. Examination of exsiccati of Kabát and Bubák (FIG. 2, J) and Stares shows that they do not differ significantly from the California fungus.

4. *Ramularia Lathyri* Hollós. This fungus was described on *L. hirsutus* in Hungary in 1910 (**24**). The conidiophores were fasciculate and hyaline, 40–50 × 3 μ; the conidia cylindrical or sometimes obtuse, catenulate, continuous or 2-celled, hyaline, 16–20 × 4–5 μ. The binomial *R. Lathyri* Hollós was used by Siemaszko (**47**) for a fungus on *Lathyrus roseus* Stev. in the Caucasus in 1923.

5. *Ramularia roscola* Bubák et Vleugel. This species was applied to a *Ramularia* on *L. maritimus* (L.) Bigel. in Sweden in 1914 (**9**). The leaf spots possessed definite margins and the tufts of conidiophores were pink. Conidiophores were fasciculate and 28–56 × 2.5–3 μ. Conidia were catenulate, hyaline; microconidia

were continuous, 9–13 × 2.5–3.5 μ; macroconidia cylindrical, 19–28 × 2.5–4 μ, continuous to uniseptate. This binomial was applied by Cash to a collection made by Anderson (Alaska Fungi No. 994; FIG. 2, L) on *L. palustris* in 1923. Examination by us of Anderson's specimen showed that it differed in no significant particular from the other fungi under consideration.

6. *Cladosporium album* Dowson. This binomial was applied in 1924 (**14**) to a fungus that caused leaf spots of *Lathyrus odoratus* in England. The types of lesions produced and the characteristics of the fungus on the host and in culture agree with those of the California pathogen. Apparently in classifying this fungus greater emphasis was placed on its appearance in culture than on the host, and the taxonomic importance of color was minimized. Leaf specimens were stated to have been independently examined by Miss E. M. Wakefield and Dr. J. Ramsbottom and considered to be an unusual form of *Ramularia*. Cultures were examined by Prof. F. T. Brooks and said to resemble *Cladosporium herbarum* except in color. Examination by us of Dowson's exsiccati (Coll., Surrey, England by Dowson, Oct. 23, 1924) showed that *C. album* (FIG. 2, K) did not differ from Fuckel's specimen or the California material. Good (**19**) minimized the differences between *C. album* and *C. fulvum* Cke. in culture prior to sporulation. Because *fulvum* mutated to a hyaline form, he also felt that color was of little fundamental importance. There are marked differences between colonies of the *Lathyrus* fungus and *C. fulvum* in growth rate and habit, maximum size attained, and color (FIG. 1, F–H), as well as in morphology (FIG. 2, N, O).

There are close similarities of spore formation in culture by the sweet-pea fungus [Fig. 50 of Dowson (**14**) and Fig. 7 of Good (**19**)], established *Ramularia* spp. [Figs. 9–12 of Laibach (**31**), Fig. 20 of Hughes (**26**), and Fig. 9 of Gregory (**20**)], and established *Cladosporium* spp. [FIG. 2, O of the present paper], particularly when they are examined under low magnification. However, careful studies of *R. deusta* under high magnification reveal a tendency toward fasciculation in culture, and the spores tend to form more readily into long chains than in clusters as in *Cladosporium*. The character of the scars and the shape of the conidio-

phore tips as well as the connections between conidia are quite distinctive (FIG. 2, N, O). Morphology of the fungus in culture obviously cannot be used alone for identification of these genera. Because of these considerations, the designation of the *Lathyrus* organism as a *Cladosporium* obscures its true affinities both as an entity and as a pathogen.

Seymour (**46**) questioned "the accuracy of the determination" of the reported *C. album* in North America. Following a discussion with the senior author, Savile (**11**) stated that this fungus should be regarded as a *Ramularia* rather than a *Cladosporium*.

Classification of many Fungi Imperfecti is based in large measure on their sporulation on the host. Some of the Melanconiales, for example, produce no acervuli in culture and cannot be correctly identified unless observed in their parasitic state. Of pathologists and mycologists who examined the white-blight fungus on the host for us, several placed it in *Ramularia* or *Ovularia* but no one in *Cladosporium*. The older name, *Ovularia deusta*, applied to the same fungus more nearly expresses its relationships.

Dowson (**14**) emphasized that the indefinite margins of the leaf-spot on sweet pea were atypical for *Ramularia*, but were known for *C. fulvum* on tomato. Similar diffuse leaf spots are caused by *R. Vallisumbrosae* on narcissus (**20**) and *R. cyclaminicola* on cyclamen (**4**). The form of *Ramularia deusta* on perennial pea consistently produces definite dark-margined leaf spots, as apparently is the case on wild *Lathyrus* spp. Possibly a diffusible toxin is formed during invasion of sweet pea but not of other *Lathyrus* spp. Spots on leaves of sweet pea often lose their diffuse character when pressed and dried, showing necrotic areas with almost as sharp a margin as those on perennial pea.

The binomial *C. album* has been commonly used in Europe and North America [*e.g.*, **1, 3, 5, 12, 19, 21, 23, 32, 37, 40, 41, 45**; Guterman (Cornell University Department of Plant Pathology herbarium No. 24750); Cash (Forsell and Ramsey in Washington, October 29, 1943, U. S. Dept. Agr. Mycol. Dis. Surv. herbarium)].

7. *Erostrotheca multiformis* Martin and Charles. This name was applied (**37**) in 1928 to a fungus that was thought to be the perfect stage of *C. album*. Good (**19**) has expressed doubt about

this relationship because of differences in morphology and colony size between this fungus and *C. album* in culture.

There are several lines of evidence that lead to the conclusion that *E. multiformis* is not connected with *C. album*. The results of Dowson, Good, and the writers are in agreement that the causal organism is extremely slow-growing, producing colonies about 10 mm. in maximum diameter from single conidia (FIG. 1, G, H). A single-spore culture is not macroscopically evident for at least a week. *Erostrotheca* was illustrated and described (**37**) as rapidly producing luxuriant zonate colonies about 40 mm. across and forming the perfect stage in 6 days.

Evidence is lacking in the original paper (**37**) that any of their 7 "stages" of the fungus in culture (*Cladosporium, Hormodendron, Ovularia, Haplaria, Pseudofumago, Pseudosaccharomyces, Erostrotheca*) was tested for pathogenicity. Because of the uncertain status of these various forms, it is not clear that the true pathogen was even represented among the imperfect forms observed in culture or that they had any connection with the sexual stage. Examination of leaves from their material [3] collected on Long Island, New York, on May 24, 1927, and labeled "original culture made from *Cladosporium* stage on this material" has established the identity of their fungus, *C. album* (FIG. 2, M), with *Ramularia deusta* in California. Martin and Charles (**37**) stated that specimens from Pennsylvania, Massachusetts, and from Dowson in England proved to be the same fungus (*C. album*) as their New York material. An ascigerous fungus was obtained by them in one isolate out of 15, and this culture was used in all studies. It seems probable that the *Erostrotheca* overgrew and suppressed the slow-growing *C. album*. Slow development of small colonies in culture is usual for *Ramularia* and *Ovularia* (**20, 26, 29**) but not for *Cladosporium*. We have cultured various *Ramularia* and *Ovularia* species (*e.g., Ramularia cyclaminicola* Trel. from cyclamen, *R. decipiens* Ell. & Ev. from Rumex, *R. pastinacae* Bub. from parsnip, *R. lactea* (Desm.) Sacc. from Viola, *R. cynarae* Sacc. from artichoke, *R. primulae* Thüm. from Primula, *Ovularia* sp. from vetch)

[3] Specimen obtained through the courtesy of Dr. W. D. McClellan and Dr. P. Brierley.

and all produced slow-growing colonies similar in appearance to *R. deusta*.

Erostrotheca is a member of the Hypocreales. All *Ramularia*, *Ovularia*, or *Cladosporium* spp. known to have a perfect stage are in Mycosphaerella of the Sphaeriales. We have been unable to find any record of observation or collection of the genus *Erostrotheca* since its erection.

In order to clarify this situation we have for some years searched for the perfect stage of the two strains of *Ramularia deusta* in California. Cultural studies and examination of field material at various seasons have failed to provide more than a suggestion that one exists. During the winter months the *latifolius* form frequently produced in substomatal cavities of leaves sclerotia-like structures which bore conidiophores on the upper surface (FIG. 2, C, G). These structures resemble those described (**20**) for *Ramularia Vallisumbrosae* Cav., and similarly may represent immature perithecia. On the other hand, it is equally possible that these are sclerotia functioning solely for aestivation as reported for *R. onobrychidis* (**26**). Structures that were perhaps spermagonia have been reported (**20, 26**) without clear genetical connection with these two fungi. Similar structures resembling spermagonia were present on old perennial pea leaves in winter in California, but it was not demonstrated that they were a stage of *R. deusta*. Their presence suggests the possible existence of a perfect stage of the *Mycosphaerella* type. Good (**19**) reported structures for the sweet pea strain in culture that also suggested spermatia and perithecial initials. Laibach (**31**) and Killian (**29**) reported the formation of sclerotia in numerous *Ramularia* spp. Production of "sporodochia" by the sweet-pea fungus (FIG. 2, A) is consistent with their demonstrated formation (**20, 26, 29**) by various *Ramularia* spp. during the summer months. These similarities of the *Lathyrus* fungus to known Ramularias support the close relationship to that genus presented in this paper.

In consideration of these facts, the name *Erostrotheca multiformis* is set aside pending evidence that it is the perfect state of *C. album*.

8. *Hyalodendron album* (Dowson) Diddens. *Cladosporium al-*

bum was transferred to this genus by Diddens (**13**) at the time it was erected for a saprophytic wood-pulp fungus. *C. album* was studied in culture and considered to be better included in *Hyalodendron* than left as a hyaline *Cladosporium*. There is no evidence that the fungus was studied on its host. This transfer is rejected because it obscures the relationship to other leaf-spotting *Ramularia* and *Ovularia* spp. and would impede practical identification of the fungus, and because *Ovularia deusta* has nomenclatural priority.

9. *Ramularia deusta*. The binomial, *Scolicotrichum deustum* Fuckel, has priority over other names applied to this fungus. Because Fuckel described and correctly placed species in the moniliaceous genus *Ramularia* and the dematiaceous genus *Scolicotrichum* in his Symbolae Mycologicae, it is inexplicable why the *Lathyrus* fungus was placed in *Scolicotrichum*. Certainly the exsiccati and his description leave no doubt that the fungus he named *S. deustum* was the *Ramularia* treated in this paper. This error was recognized by Saccardo and the fungus transferred to *Ovularia* in 1886.

The basis for distinguishing *Ovularia* from *Ramularia* is imperfectly defined and there is question whether they should be separated at all (**26**). *Ramularia* was erected by Unger in 1833 and *Ovularia* by Saccardo in 1880, and should a future monographer combine these genera, the older name would be retained.

In the morphological characters proposed for separating these genera, our fungus more nearly approaches *Ramularia* than *Ovularia*. Ramularias are considered to form spores in long chains as does our fungus, whereas Ovularias bear them singly or in short chains. Numerous Ramularias have been shown (**20**, **26**, **29**, **31**) to develop sclerotia, as does our fungus (FIG. 2, C, G), while these structures are absent or inconspicuous in *Ovularia*. The pathogen is nearer to *Ramularia* than to *Ovularia* because of its cylindrical spores. The degree of roundness of the spores has been found to vary with fresh *vs.* dried material, on host *vs.* in culture, and on the host under dry *vs.* moist conditions (FIG. 2, D, E) as reported for other Ramularias (**20**, **26**). The germination of conidia is shown in figure 2, F.

Under moist conditions, some Ramularias tend to form in the co-

nidial apparatus elongate structures [4] that have been regarded (20) as scolecospores, but can as logically be interpreted as phialides of the conidiophores. They are much less common in *Ovularia* than *Ramularia*. The diverse collections of *R. deusta* examined showed all gradations of development of these structures (FIG. 2, A–C, H–M).

Because of these considerations, the fungus is transferred from *Scolicotrichum* to *Ramularia*. An emended description and the synonomy of this pathogen are as follows:

Ramularia deusta (Fckl.) n. comb.

Synonomy:

Scolicotrichum deustum Fuckel, 1869.
Ovularia deusta (Fuckel) Sacc., 1886.
Ovularia deusta Sacc., 1887 (6).
Ramularia Galegae f. *Lathyri* Fautrey, 1898–99 (28).
Ramularia Galegae Sacc. f. *Lathyri* Ferraris, 1906.
Scolecotrichum deustum Fuckel, 1907 (34).
Ramularia Lathyri Ferraris, 1910 (28).
Ramularia Lathyri Hollós, 1910.
Ramularia Lathyri (Ferr.) Bubák, 1916.
Ramularia roscola Bubák et Vleugel, 1916 (9).
Ramularia Galegae f. *Lathyri* Fautr. apud Ferraris, 1923 (28).
Ramularia Lathyri (Fautr.) Ferraris, 1923 (28).
Cladosporium album Dowson, 1924.
Hyalodendron album (Dowson) Diddens, 1934.

Nomen ambigum: *Erostrotheca multiformis* Martin and Charles, 1928.

Conidiophores densely fasciculate, typically unbranched, slender, hyaline, continuous or with several septa, highly variable in length, 20–60 × 3–4 μ [usually 50 × 3–4 μ; under moist conditions develop elongate structures (conidiophores or "scolecospores") which extend the total length up to 200 μ], bear terminal conidia, and become geniculate with age. Conidia cylindrical, with tapering ends, continuous or 2-celled, hyaline, minutely echinulate, 2–28 × 1–5.5 μ, borne in acropetalous fashion, forming short chains which may be branched.

[4] Since this paper was submitted for publication, my attention has been called to an article by Dr. W. C. Moore in which a similar situation in *Ramularia primulae* is discussed (Trans. Brit. Mycol. Soc. 25: 208–209. 1941).

PHYSIOLOGIC FORMS

Slight differences in colony appearance between the sweet pea and perennial pea isolates and the differences in color of sporulation and symptom expression on these hosts suggested the possibility that biologically specialized races of the fungus were involved in these two diseases. To test this possibility, sweet peas and perennial peas grown in pots were cross inoculated by atomizing them with spore suspensions of the organisms produced in culture. Although only one such test was made, the results indicated that the two isolates were biologically distinct. Typical leaf spots and stem lesions were obtained on *L. latifolius* only with the isolate from that host (FIG. 1, B). Likewise, infection of *L. odoratus* occurred only when the sweet pea isolate was used. The controls were not infected. It is concluded that the *Ramularia* leaf spots of sweet pea and perennial pea are caused by distinct races of *Ramularia deusta*.

Inoculation data available, including that reported by Good (**19**), plus the known host specificity of Ramularias and Ovularias, indicate the existence of a number of physiologic forms of *R. deusta*. It would seem best to designate these as formae of the morphologic species, *R. deusta*, as they become known through inoculation tests. Accordingly, we propose two forma names to distinguish between the strains which attack sweet pea and perennial pea: **Ramularia deusta** f. **odorati** n. f. and **Ramularia deusta** f. **latifolii** n. f., pathogenic and causing leaf spots upon *Lathyrus odoratus* and *Lathyrus latifolius*, respectively.

DISCUSSION

In an admittedly artificial system of classification such as that of the Fungi Imperfecti, the convenience and accuracy of identification of specimens should be a major consideration. It is important to pathologists that an imperfect fungus be so placed that other workers will readily recognize the relationship of their organism to it. The nomenclatural situation discussed in this paper shows the confusion that results from parallel usage of different names for the same organism. A similar situation is discussed elsewhere (**4**) for *Ramularia cyclaminicola* Trel. The established

basis for classification of fungi of this type is their morphology on the host with only secondary consideration to cultural characters. The fungus considered here is a *Ramularia* in its parasitic state and is therefore transferred to that genus.

The spore measurements of the majority of descriptions and specimens that we have examined fall within the fairly consistent range of 2–20 × 1–5.5 μ. However, descriptions of two of the binomials here considered (*Ramularia Galegae* Sacc. f. *Lathyri* Ferraris and *Ramularia roseola* Bubák et Vleugel) have somewhat larger spores. Despite this difference, the former has been considered (**9, 28**) as synonymous with the smaller spored *R. Lathyri* Hollós. Because this range includes measurements of spores produced on culture media, numerous *Lathyrus* species, and under different moisture conditions (**20, 26**), the variation in conidial size is not regarded as significant. The range of variation (2–28 × 1–5.5 μ) of all collections of *R. deusta* discussed here is small compared with that demonstrated (**20, 26**) for *R. Vallisumbrosae* (3–44 [scolecospores up to 105 μ] × 2–4 μ) and for *R. onobrychidis* (7–42 × 2.7–6.3 μ).

Other *Lathyrus* pathogens discussed here are considered to be the same as Fuckel's *deusta*, which is here transferred to *Ramularia*. This specific name has the advantage of almost continuous usage since 1869. The use of the forma names, *odorati* and *latifolii*, to denote host specificity permits retention of the morphologic species *R. deusta*, while distinguishing between its physiologic forms.

SUMMARY

Evidence is presented that certain *Lathyrus*-infecting fungi of the genera *Scolicotrichum*, *Ozularia*, *Ramularia*, *Cladosporium*, and *Hyalodendron*, including the pathogens causing white blight or white mold of sweet pea and a previously undescribed leaf spot of perennial pea, should be designated as *Ramularia deusta* (Fckl.). Other specified binomials, including the commonly used *Cladosporium album* Dowson, become synonyms.

It is concluded that the relationship of the Ascomycete, *Erostrotheca multiformis* Martin and Charles, to the sweet pea Hyphomycete has not been demonstrated, and it is improbable that they

are states of the same fungus. This name is therefore set aside, pending evidence of such genetical connection.

The situation discussed here illustrates how parallel use of different names for the same fungus, arising from incomplete information of the pathogen's development on the host and in culture, results in confused nomenclature and obscured relationships.

Ramularia deusta is widespread over the temperate and subtropical world on 9 species of *Lathyrus*, including the cultivated *L. odoratus* and *L. latifolius*. Physiologic specialization is probably of common occurrence in *R. deusta*. Two cases are recognized here by the proposal of two new forma names, i.e., *R. deusta* f. *odorati* n. f. for the pathogen of *Lathyrus odoratus*, and *R. deusta* f. *latifolii* n. f. for the pathogen of *L. latifolius*.

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DESCRIPTION OF FIGURES

FIG. 1. *Ramularia* leaf spot of perennial pea. A, C, Natural infections of leaves and a stem, respectively. B, Infections resulting from atomizing with spores from a pure culture. D, E, Magnified conidiophore tufts on leaves. F, Twenty-day-old cultures of *Cladosporium fulvum* on potato dextrose agar,

grown from single conidia in light at laboratory temperature. G, Twenty-day-old cultures of *Ramularia deusta* f. *latifolii* comparable to F. H, Sixty-day-old cultures of *R. deusta* f. *latifolii*.

FIG. 2. Camera-lucida drawings. A–G, N, *Ramularia deusta* (Fckl.) n. comb. collections on perennial pea. A, B, Conidiophores developed in summer, A showing a "sporodochium." C, Elongated conidiophores developed in winter from a sclerotium. D, Conidia developed under dry conditions. E, Conidia developed under moist conditions. F, Germinating conidia on water agar. G, Sclerotium developed in leaves in winter. H, *Scolicotrichum deustum* Fckl. on *Lathyrus tuberosus* from Fuckel's Fungi rhenani exsiccati No. 2206. I, *Ovularia deusta* (Fckl.) Sacc. on *L. sylvestris* from Sydow's Mycotheca germanica No. 2224. J, *Ramularia Galegae* Sacc. f. *Lathyri* Ferr. on *L. odoratus* from Kabát and Bubák's Fungi imperfecti exsiccati No. 791. K, *Cladosporium album* Dowson on *L. odoratus* from Dowson's collection, Surrey, England, Oct. 23, 1924. L, *R. roseola* Bub. & Vleug. on *L. palustris* from Anderson's Alaska Fungi No. 994. M, *C. album* on *L. odoratus* from Charles and Martin's collection, North Baldwin, Long Island, N. Y., May 24, 1927, labeled *Erostrotheca multiformis* Martin & Charles. N, *R. deusta* from culture grown on water agar plus cold-sterilized ground aster stems, in light at laboratory temperature. O, *C. fulvum* from cultures comparable to N.

DESCRIPTIONS OF TWO LUMINOUS TROPICAL AGARICS (DICTYOPANUS AND MYCENA)

E. J. H. Corner *

(with 3 figures)

The agarics here described were found by Dr. Y. Haneda and myself in the Singapore Botanical Gardens during the years 1943–1945 when he had charge of the Raffles' Museum. Dr. Haneda had been interested in bioluminescence for some years and he brought to my notice his publications, in Japanese, on luminous fungi from Palao Island in the Pacific. We discovered several of these fungi in Singapore and this paper is the outcome of our preliminary investigations. It was written towards the end of 1944, and in 1949 one copy came through Dr. Haneda in Japan to Dr. A. H. Smith, who kindly returned it to me for revision. One new species, *Dictyopanus luminescens*, belongs to the luminescent alliance of *Panus stypticus* and the other is a new variety, *Mycena rorida* var. *lamprospora*, which presents the hitherto unknown case, according to a verbal communication from Dr. E. C. Wassink, of luminous discharged spores without any part of the fruit-body itself being luminous. Dr. Wassink is arranging for the translation of the copies of Dr. Haneda's papers, which contain much of great interest to students of bioluminescence in general.

I have much pleasure in expressing my gratitude to Dr. Haneda, Dr. Smith, and Dr. Wassink for their cooperation, but it is with great regret that I have to record at the same time the loss of all the manuscript that Dr. Haneda himself prepared during his tenure of office in Singapore.

Dictyopanus luminescens sp. nov. (Figs. 1 and 2)

Fructus toto noctilucens, minutus, lateraliter stipitatus, gregarius, albus demum sordide flavescens vel alutaceus.

* Botany School, Cambridge, England.

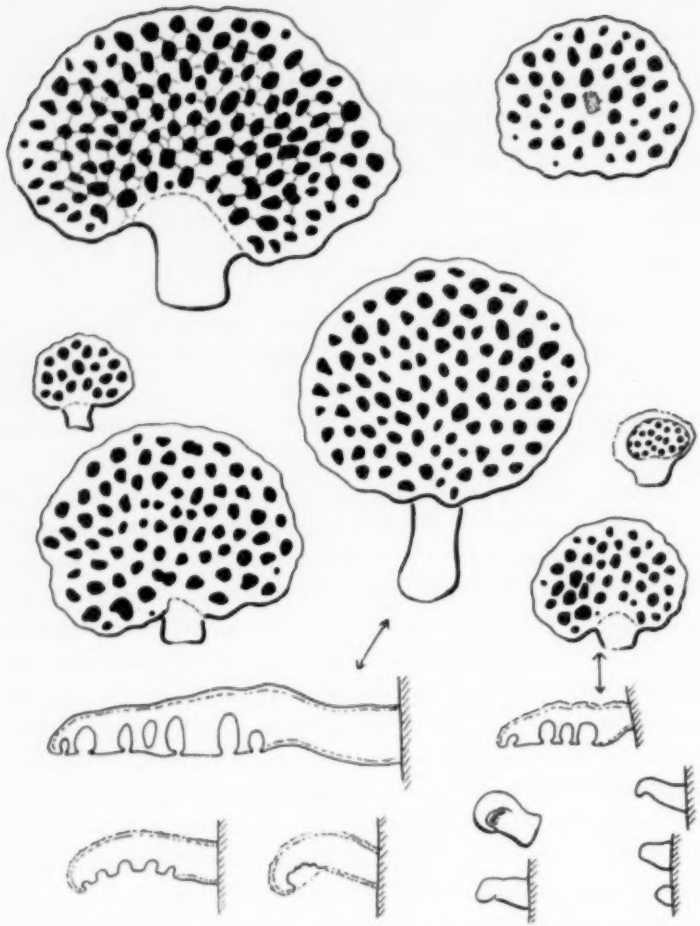

FIG. 1. *Dictyopanus luminescens;* fruit-bodies seen from below, with developmental stages in section, × 15.

Pileus 2–4 mm. latus, convexus demum explanatus, reniformis, toto minute cretaceo-pruinosus, opacus, siccus, demum cute minute fracto: margine integro, incurvato demum explanato.

Stipes 0.1–1.3 × 0.1–0.5 mm., brevis, lateralis (raro subcentralis, ut pileo cretaceo-pruinosus.

Tubuli ad 350 μ longi, 200–250 μ lati, paullum radiati, distincti, nec decurrentes nec lamellosi: pori 70–150 μ lati, constricti, cretaceo-albi: dissepimenta crassa, 50–80 μ lata, tumida, demum minute fracta.

Caro 100–350 μ crassa, alba, sicca, paullum coriacea.

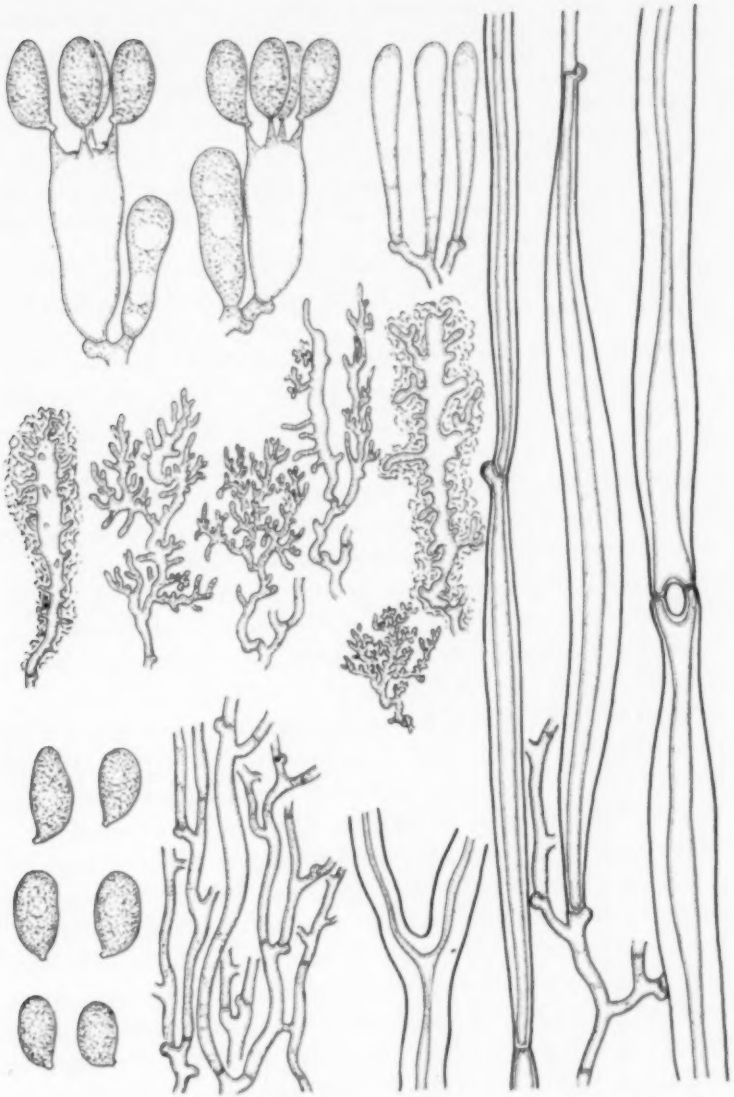

FIG. 2. *Dictyopanus luminescens;* spores and basidia, × 1000; hyphae from the pileus and the base of the stem (thick-walled), × 500; cheilocystidia (clavate) and spiculiferous hyphal ends from the surface of the pileus, × 500.

Sporae 9–13 × 5–6 μ, vulgo 10–11.5 × 5.5 μ, vel minores 7.5–9 × 4–4.5 μ, albae, ellipsoideae, elongatae, leves, aguttatae, parietibus amyloideis.

Basidia 20–28 × 9–10 μ, vel minora 18–25 × 7.5–8.5 μ (dein, sporis minoribus), strato continuo instructa: sterigmata 4, 5–6 μ longa.

Cheilocystidia 30–55 × 7–10 μ, subclavata, tenuiter tunicata, ad margines tubulorum: pleurocystidia nulla.

Hyphae simplices, 1.5–4 μ latae, furculatae, ad superficies fructi angustissimae, dense et intricater coralloideo-ramosae vel spiculiferae, plerumque granulis hyalinis dense incrustatae: in stipite aliae cellulis inflatis 100–250 × 6–20 μ et crasse tunicatae, parietibus ad 7 μ crassis.

Hab. ad folia palmarum emortua (*Rhapis, Pinanga, Arenga*) in silvis: Malaya (? Asia tropicali et Australasia): *typus*, leg. Corner, Singapore Botanical Gardens, 1944.

This is a common Malayan fungus, to be found every rainy season on the dead leaves of palms and, in the Singapore Botanical Gardens, particularly on the dead persistent sheaths of the erect stems of *Rhapis flabelliformis*. Dr. Haneda showed me the bright green phosphorescence of the whole fruit-body and told me that it was probably the fungus recorded as *Polyporus rhipidium* Berk. from the Bonin Islands by Kobayashi (**1937**).

The genus *Dictyopanus* has recently been revised by Singer (**1945**). *D. luminescens* is near to *D. Copelandii*, on dead grasses in the Philippines, and to *D. pusillus* of the New World, but it is characterized by the short stem, the never lamellate pores, the large spores and, perhaps, the luminescence. Singer regards *Polyporus rhipidium* as a variety of *D. pusillus* (= *Polyporus pusillus* Lev.). Now, in addition to Kobayashi's record of luminescence in *P. rhipidium*, Wassink (**1948**) has recorded the mycelium of a strain, so named, from Australia as luminous in culture in Holland. Following Berkeley's original suggestion that *P. rhipidium* resembled *Panus stypticus*, Wassink realized the affinity of the two from their luminescence, but it is possible that both his culture from Australia and Kobayashi's fungus from the Bonin Islands were the large-spored *D. luminescens*, the spore-contents of which shrink on drying to a small lump about the size of the spores of *D. pusillus* (3.5–5 × 1.5–2.5 μ). The spores of *D. Copelandii* are intermediate, 6.8–7.8 × 3.5–4.2 μ, but the pores of this species are characteristically lamellate at the stem-apex.

Dictyopanus differs from *Panellus*, in which genus *P. stypticus* is now placed, merely in the poroid hymenium, but this feature is

not constant in the American *D. pusillus*, which may be more or less poroid-lamellate and so approaching the state of *P. stypticus* with fine reticulations at the base of the gills. A revision of the tropical species of the two genera will probably show the necessity of merging them into one, for which luminescence may be a feature.

Mycena rorida (Fr.) Quél. var. **lamprospora** var. nov. (FIG. 3)

A typo differt lamellis principalibus paucioribus 9–14: sporis minoribus 5.7–10 × 3–4.7 μ, noctilucentibus: basidiis semper 4-sporigeris: cheilocystidiis saepe irregulariter lobatis: pileocystidiis grandis, 25–65 × 15–40 μ, clavato-pyriformibus vel subglobosis.

Hab. ad folia et ramulos emortuos in silvis tropicalibus: Asia (Malaya, Ceylon, Rabaul), America (Brazil).

Typus: leg. Corner, Singapore Botanical Gardens, 14 Nov. 1944.

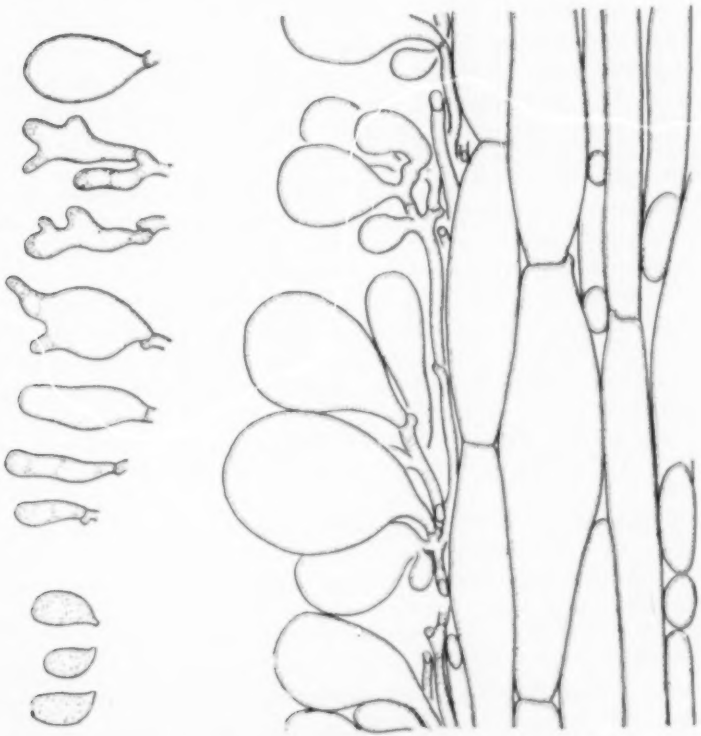

FIG. 3. *Mycena rorida* var. *lamprospora*; spores, × 1000; cheilocystidia and pileocystidia with the adjacent tissue of the pileus, × 500.

Pileus 3–12 mm. wide, *persistently convex or campanulate*, varying subumbonate to subumbilicate, dry, hygrophanous, *white to pale fuscous or fuscous umber, pruinose with minute fuscous brown particles* scattered toward the white margin, crowded over the deeply colored disc, *striate;* margin slightly incurved at first, finally subcrenulate.

Stem 1–2.8 cm. × 0.5–0.8 mm., slightly thickened downward, hyaline pellucid, *viscid, often thickly glutinous toward the base, white, becoming pale fuscous then yellowish fuscous from the base* upward, the base abrupt and villous within the gluten.

Gills *broadly adnate to decurrent, subdistant,* 2–3 ranks, 9–14 primaries 1.3–3.5 mm. wide, *white* or pale fuscous at the base, often subcostate, with fuscous edge in the Brazilian specimens.

Flesh very thin, hygrophanous, concolorous: odor none.

Spores 5.7–10 × 3–4.7 μ, white, *luminous* when damp and fresh, pip-shaped oblong, aguttate, thin-walled, smooth, the contents cloudy, the wall amyloid.

Basidia 18–30 × 6.5–9 μ: sterigmata 4, 3.5–4 μ long.

Cheilocystidia 15–36 × 6–18 μ, forming a narrow sterile edge, clavate to subventricose or subglobose, often irregularly lobed but not digitate, thin-walled, smooth, with pale brown sap in the Brazilian specimens: pleurocystidia none.

Pileocystidia 25–65 × 15–40 μ, clavate-pyriform or subglobose, thin-walled, smooth, with deep umber sap, proliferating from the narrow stalk, set in clusters forming a disrupted palisade, more or less continuous over the center, the stalks 2–3 μ wide.

Caulocystidia –30 × 2–4 μ, as simple scattered subcylindric and irregular processes into the gluten from the narrow superficial hyphae of the stem, more abundant at the stem-apex and compacting into the hymenium: at the base of the stem, elongating into aseptate or sparsely septate hyphae –300 × 2–4 μ, tapering to slender points, projecting at right angles from the stem into the gluten, and with pale brownish ocher, slightly thickened, glutinous walls.

Hyphae in the stem longitudinal, clamped, the cells 100–400 × 8–22 μ, or – 35 μ wide at the base of the stem, up to 1100 μ long in the Brazilian specimens, the septa broad and transverse, without interweaving hyphae: colorless at first, but the walls thickening slightly and becoming pale brownish from the base upward.

Hyphae of the pileus as in the stem but with colorless walls: with a narrow pseudoparenchymatous layer, 1–3 cells thick, just below the palisade of pileocystidia, the cells 30–150 × 10–30 μ, with fuscous umber sap: in the gills, as in the pileus, not pseudoparenchymatous, the subhymenium c. 8 μ thick and composed of narrow hyphae 2–3.5 μ wide.

Hab. on dead leaves and twigs in the forest: tropical Asia (Ceylon, Malaya), Australasia (Rabaul), and America (Brazil).

This species can be found in Malaya every fungus season, but it is usually scattered and cannot be considered common. It is remarkable among luminescent fungi because only the fresh damp spores, as seen round the base of the stem on the stick or leaf, are luminous. Yet, I am unable to find any other distinctions from the well-known European and North American *M. rorida* than those given in the diagnosis, and they do not suggest a different species. For this reason, and because it is always advisable to check very carefully tropical records of temperate species, I have given a full description of the variety as I have found it myself in Malaya and Brazil.

Concerning the gills, Kühner gives 12–21 primaries and Smith 14–18 for typical *M. rorida*. Malayan specimens had 9–14 and the Brazilian 10–12.

Regarding the spores, there would seem to be a better distinction, but when analyzed in detail no qualitative difference appears. Thus, there are the following reliable records of the size and number of the spores and of the size of the basidium:

Spore-size μ	Number per basidium	Size of basidium, μ	Authority
13–14.5 × 6.6–7	2	—	Josserand, France
10–13 × 3.5–5.7	2	20–34 × 5–8	Kühner, France
10–15 × 4–5	2	30–36 × 5–6	Ricken, Germany
10.5–15 × 4–5.7	2	—	Corner, England
9–12 × 4.5–6	2	26–30 × 5–7	Smith, U.S.A.
8–10 × 4–5	4	—	Smith, U.S.A.
8–11 × 4	?	—	Rick, Brazil
7–10 × 3–4	?	—	Petch, Ceylon
8–10 × 4–5	4	23–30 × 7–8.3	Corner, Malaya
6.5–9 × 4–4.5	4	18–23 × 7–9	Corner, Malaya
5.7–8.5 × 3.5–4.7	4	18–24 × 6.5–8	Corner, Brazil

Now, on analyzing these figures in the way proposed in my paper on the connection between spore-size and number and basidium-size (**1948**), one finds the following relations:

For dispores,

Average spore-size 12.20 × 5.20 μ, volume 172.8 c.μ

Average basidium-size 29.3 × 6.0 μ, volume 575.1 c.μ

For tetraspores,

Average spore-size $7.95 \times 4.28\ \mu$, volume 76.25 c.μ

Average basidium-size $22.7 \times 7.63\ \mu$, volume 503.6 c.μ

The dispore has, thus, *2.27* times the volume of the tetraspore. Assuming a width of $4.28\ \mu$ for the tetraspore, a dispore-basidium should be able to produce four spores of the size $9.32 \times 4.28\ \mu$, which is appreciably longer than the observed average, though agreeing better with the maximum mean values of $9.0 \times 4.5\ \mu$ found by Smith and myself for the tetraspores. However, the basidia of the dispores are larger than those of the tetraspores, apparently, having 1.14 times their volume. Allowing for this correction in calculating the volume of the tetraspores derivable from the dispore-basidium, one finds an average calculated tetraspore of size $7.89 \times 4.25\ \mu$, which agrees well with the observed average of $7.95 \times 4.25\ \mu$. Hence, I conclude that the 4-spored state of *M. rorida* differs from the 2-spored not only in this number of spores but in having slightly smaller basidia.

Regarding the luminescence, Dr. Haneda first recorded this in the specimens identified as *Omphalia* sp. from Rabaul (**1942**), and together we observed the same brilliant greenish luminescence of the fallen spores in Singapore. I surmise that the Ceylon specimens described by Petch as *M. rorida* and the Brazilian specimens found by myself in Rio de Janeiro and in Manaus had also this luminiscence, though I regret that, owing to the hasty conditions under which I managed to snatch a little time for mycology in Brazil, I forgot to check this point. It is not unlikely, indeed, that the temperate *M. rorida* itself may have luminous spores, for luminosity of various parts of the fruit-body is considered as authentically proved for some 15 species of *Mycena*, according to Wassink. On the other hand, *M. rorida* may have a luminous variety as *Panus stypticus*.

M. rorida is, thus, interesting from the distributional point of view. As a whole it appears to be cosmopolitan, mainly 2-spored in Europe, 2–4-spored in North America, and 4-spored in the tropics. In the viscid stem and the palisade of clavate cells on the pileus, making it truly pruinose, the species indicates a distinct section of the genus and, as I have shown in a forthcoming

monograph on clavarioid fungi, such wide-spread distribution of sections, microgenera and, indeed, individual species seems to be the rule among basidiomycetes: the nearest allies of many temperate species are tropical, and *vice versa*.

SUMMARY

Dictyopanus luminescens, from Malaya, has wholly luminous fruit-bodies. Specifically it is characterized by the large spores 9–13 × 5–6 μ. The records of luminescence in *Polyporus rhipidium* from the Bonin Islands and from Australia may refer to *D. luminescens*.

Mycena rorida var. *lamprospora* has smaller spores, four per basidium, than the typical 2-spored state of the species, and these spores, when fresh and damp, are luminous. The variety is definitely recorded from Rabaul and Malaya, very probably from Ceylon and Brazil, and may be pantropical.

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PULLULARIA AS A CAUSE OF DETERIORATION OF PAINT AND PLASTIC SURFACES IN SOUTH FLORIDA [1, 2]

ERNEST S. REYNOLDS

(WITH 2 FIGURES)

GENERAL ECONOMIC SIGNIFICANCE OF PULLULARIA

In recent years *Pullularia* (**5, 6**), usually under this generic designation or often under the older name, *Dematium pullulans* DeBary, has been reported with increasing frequency from various parts of this country and from other parts of the world. In the older references especially, it was often described as a saprophytic mold with no particular rôle assigned to it, but more recently it has been reported as an important if not a primary element of the sooty-molds growing on "honey-dew" in Europe (**30, 45**) and New South Wales (**14**).

As an organism associated with deterioration of plant parts and plant products *Pullularia* has been reported sufficiently often to justify considering it of considerable economic significance. In some references it has been indicated as a cause or a cooperating cause of disease or injury in plants (**1, 4, 15, 16, 27, 31, 49**) or of damage to fruit (**10, 11, 12, 13, 46**). It appears in general to be "saprophytic or weakly parasitic" as stated by Bennett (**5**) in his studies of its effects on wheat.

As a flax retting organism it is reported from the Australian Commonwealth (**2, 21**); and as an element of importance in the

[1] Contribution No. 36, Moisture- and Fungus-Proofing Research Program, Bureau of Ordnance, Department of the Navy, and Contribution No. 40 from the Marine Laboratory, University of Miami.

[2] Presented at a joint session of the Microbiological Section of the Botanical Society of America and the Mycological Society of America, September 13, 1948. The opinions expressed herein are those of the author and not necessarily those of the organizations involved.

pulp and paper-mill mold floras it has been reported from Finland and Sweden (**22, 25, 28, 39**), although it seems to have been reported only once as a possible member of the true paper-mold-flora (**47**, p. 289–290). As a timber blue-staining organism it is of frequent occurrence in Sweden (**7, 8, 22, 37**) and Finland (**24**). Although listed as having been isolated from "cotton hairs" it is listed also as having no "cellulose decomposing power" (**47**, p. 255). A later study (**33**) describes a black deterioration of cellulose strips by *Pullularia* alone and also in association with some other molds; but recent studies at the Quartermaster Depot in Philadelphia find no proof of cellulose decomposition by various strains of *Pullularia* including some received from Florida (**3, 36, 50**).

In Puerto Rico *Pullularia* was isolated from human skin (**9**) as a possible cause of disease, but apparently no allergenic importance has been ascribed to it (**29**). Sheep leather has been found to be spotted by *Dematium* (**19**), which along with other molds has been listed by another writer as capable of altering the structure of wool (**35**). *Pullularia* has been reported in recent listings of molds obtained from military materiel exposed to contamination in tropical and sub-tropical areas (**3, 50**) although no special rôle has apparently been assigned to it. We may conclude from the studies briefly reviewed above that *Pullularia* is of considerable general economic importance, especially in the paper-pulp industry and as a secondary or cooperating organism in injury to plants and to plant parts and products in various parts of the world, and that its importance is not confined to the sub-tropical and tropical areas.

As a significant cause of paint deterioration, *Pullularia* was originally reported in 1920 by Haenseler (**17, 18**) to be found in association with other molds on painted surfaces in New Jersey. Since that time very little attention has been given to it either by practical paint men or by mycologists.[3] It was found in the course

[3] After the preparation of this paper for presentation, a paper by Milton Goll and George Coffey entitled, "Mildew of Painted Surfaces," was published in the *Paint, Oil and Chemical Review* of August 5, 1948. These authors attribute a large part of mold deterioration of painted surfaces to *Pullularia*.

of my investigations that there was no reference to *Dematium* or *Pullularia* on paints under "Molds" or "Paints" or other pertinent headings in the Decennial Indexes of the Chemical Abstracts covering the years to and including 1936 and only twelve direct references down to that time to paint molds. During the last decade considerable activity has been shown in preparing and testing paints for resisting mold growth in general, but only occasional reference was made to *Dematium* as a paint mold. However, it appears from the most recent studies of Messrs. Goll and Coffey, and as reported in this paper, that the production of paint specifically resistant to *Pullularia* would be an important development.

PULLULARIA IN SOUTH FLORIDA

The presence and possible importance of *Pullularia* in South Florida was first called to the attention of the writer through a series of paint samples in which the green, brown, and red painted surfaces had developed numerous dark, essentially black, points. Isolations from these spots produced either pure cultures of *Pullularia* or this mold was the only dematiaceous form present. Further study of these paint samples indicated that most of the black spotting which appeared like soot or black dirt particles actually was due to spots of *Pullularia* infection. A second series of paint panels exposed a few months later at the same site developed a similar heavy infestation of *Pullularia* on certain white paints (FIG. 1, A). Approximately a year later, a commercial testing agency called attention to a serious spotting of some plastic coated glass cloth samples (FIG. 1, *C*, *D*). These also proved to be heavily infested with *Pullularia* after only a few months of exposure. In all of these cases some other molds were present on the surfaces including *Cladosporium* and species of *Penicillium*, but the major spotting was due to *Pullularia*.

A more detailed study of each of these infestations and associated pure culture work has developed data which indicate possible explanations of some of the phenomena noted in the course of this study and also suggest further necessary studies in order to gain a satisfactory understanding of some of the problems involved.

PULLULARIA ON PAINTED SURFACES

As noted before, two paint tests have been made on which *Pullularia* has developed.[4] In the first series there were ten paint types, half of the samples of each containing anti-mold additive and half without. Half of the treated samples and half of the untreated were exposed vertically in a rack standing outdoors and the other half of each was in a "stabilized natural" test chamber (**41**) which approximated 30–35° C. and 80–85% relative humidity as averages for the higher ranges. The second paint series consisted of nine interior paint types having three specimens with anti-mold additive each and three untreated, two of each three exposed outdoors and one each in the test chamber. For about two months the outdoor exposures of the first series were in a mangrove swamp and then were transferred to an open, cemented platform near the bay. During part of the day this area was partly shaded from the direct sunshine, but was neither exposed to drip from vegetation nor near any large mass of vegetation.

During the first six to eight months of exposure there were no positive evidences of fungal growth on the outdoor panels of either series of paint tests. But six months later an undetermined dark specking of one of the dark colored outdoor panels of the first series had appeared. During the third six months period most of the dark colored outdoor samples of this series had this specking, which was especially abundant near the upper ends of the panels. Some of these brown to black dots were superficial on the paint film and some were somewhat imbedded in it. Petri-dish cultures on potato dextrose agar and Czapek's solution agar made from several of these specks on different panels gave *Pullularia* growth with no other dematiaceous mold developing from the inoculum used. In some cases brown mycelial webby growth on the paint film was associated with the dots. A microscopic examination of the dot type of infestations indicated that they were composed primarily of dark-brown, oval to globular, heavy-walled spores either

[4] These were originally set up, as planned by the paint department of the Centro Research Laboratories of Briarcliff Manor, N. Y., as a part of the general program of anti-mold toxicant studies.

single or in clumps of two to several spores. Except for this mold infestation the painted surfaces showed very little effect from "general weathering" and would have been considered in good condition. None of the white to ivory panels of the first series developed the *Pullularia* infestations. The panels in the test-chamber were also free from this infestation, although *Penicillium* was present on both treated and untreated samples of the green, brown, and red paint films. It was found, however, that the panels painted with the anti-mold treated paint developed only 5 to 10% of the number of *Penicillium* patches on the untreated paints.

In the second series of paint tests all of the outdoor specimens of two white paint types, including treated and untreated paints alike, developed a heavy infestation of dark brown to black specks which gradually changed the general appearance of the paint film from white to dull gray in about two years. Specimens of the dark globular bodies inoculated into agar plates all developed *Pullularia* growth centers. From figure 1, A & B, it can be seen that the typical panel (B) with mold growth has been definitely deteriorated as compared with a typical panel of another paint in the series (A) exposed similarly but not seriously attacked by the *Pullularia*. The magnified view shown in figure 1, E, demonstrates that the paint surface itself, showing among the mold spots, withstood the general weathering conditions well and although painted with an "inside" paint, would have been satisfactory if it had not become infested with *Pullularia*.

PULLULARIA ON PLASTIC PANELS

The plastic panels which were of commercial origin were glass cloth coated, as stated by the fabricator, with "Vinylite VYNV, having Dioctyl Phthalate as the main plasticizer but with tricresyl phosphate and some amounts of another plasticizer whose chemical composition is unknown to me." Metallic pigments of various colors were used and the totally inadequate amount of ten parts per million of pyridylmercuric stearate as anti-mold toxicant was used in certain coatings. The upper, infested surfaces of two panels, one representing the toxic-treated plastic (C) and the other not treated (D), are shown in figure 1, C & D. It was

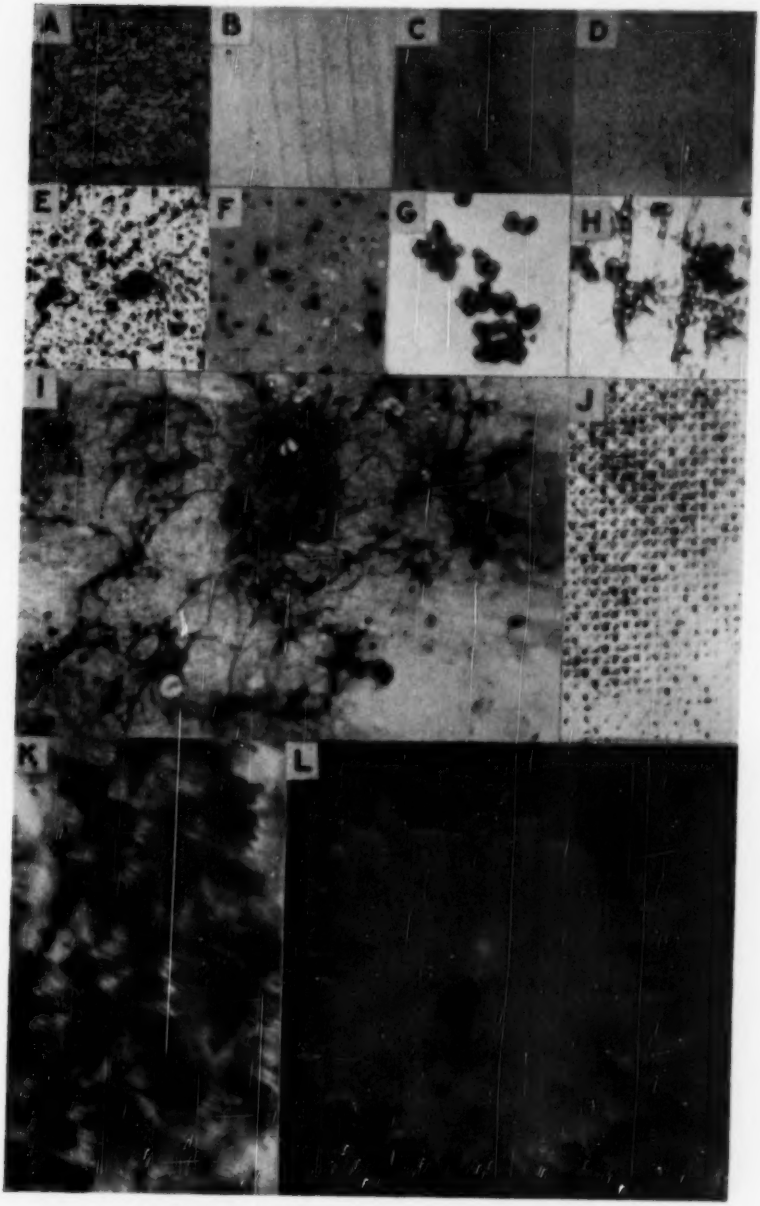

FIG. 1. *Pullularia* on painted and plastic surfaces.

evident from naked eye and microscopic examination that there was little difference between the amount of mold development on these two panels, as one would expect from the small amount of toxicant used, and that they had been well covered with the mold spots in the three months exposure period. A quantitative examination of a typical area on the non-toxic panel showed that out of a total of 62 mold colonies developed from a scraping, 53 were *Pullularia* and 9 were *Cladosporium*. An examination of ten of these plastic panels showed that all were rather heavily infested with this mold which was mainly of the black, spotted to granular type (FIG. 1, F), essentially identical with the main growth on the paint panels. In some cases the mold deterioration developed as vague, dark blotches up to four or five millimeters in diameter. On some panels the blotched condition was found also on the under side and on some, fairly large masses of the dark brown mycelium with various stages of chlamydospore formation were found on the under side where moisture could accumulate and remain for a longer period than on the upper surface, thus encouraging a more abundant vegetative growth. In still other cases a more even, very fine, black-dotted condition existed, but the microscopic appearances of the blotched areas and of this type were essentially alike (FIG. 1, G). Less frequently a mycelial web, similar to some spots on the paint panels, was intermixed with clusters of spores, on both upper and lower surfaces of some of the panels (FIG. 1, I). The majority of the hyphae in such cases had chains of chlamydospores in various stages of development (FIG. 1, H). These superficial differences seemed to be associated with differences in the plastic coating and probably with the atmospheric conditions at the time of development of the infestation. Collodion drops were placed on several spots on the upper surfaces of different panels and the partially dried film was mounted in lactophenol plus 10% O. T. as spreader. These microscopic mounts as seen in figure 1, G, H, I, give a very clear picture of the exact positional relationships of the mold on the plastic surfaces and of their detailed appearance. A microscopic examination of these plastic panels showed that while most of the spores were of the *Pullularia* irregular-globular type in small or large clusters

or less often in short chains, there were occasional stray spores of other types including *Pestalotia* and apparently *Cladosporium* [5] as the most frequent kinds. Very few dust granules were present on the upper surfaces where the black, granular infestation was most abundant.

Potato dextrose agar plates were inoculated in three to five places with mold material taken from the various types of growth described above and *Pullularia* developed in all cases. Occasional growths of other dark molds developed as well, especially *Pestalotia* and a dark mold not yet satisfactorily identified. *Trichoderma* developed on some of these plates, but did not originate from the inoculation centers except on one plate. It does not appear that any of these molds contributed largely, if at all, to the spotting of these plastic surfaces. General climatic deterioration of these plastic surfaces was noted in some cases, but the mold attacks were the primary cause of early deterioration.

The extent to which molds may penetrate and discolor resin-impregnated glass cloth is indicated in figure 1, J, K, taken from another series of tests on a different type of material, made in the high humidity chamber. The black, mixed mold growth penetrated among the strands of glass fibers to an undetermined but appreciable depth.

CULTURAL CHARACTERISTICS OF PULLULARIA

In petri-dish cultures on potato dextrose agar the *Pullularia* growth almost always starts out as a mucoid, milky, somewhat elevated, surface mass (FIG. 1, L), composed of the small, oval, colorless conidia or blastospores which multiply very rapidly for

[5] Earlier investigators, as for example Laurent (**23**) and Massee (**26**), probably were confused by the fact that frequently *Pullularia* (then designated *Dematium pullulans*) produces smooth, oval, dark, two-celled spores which are very similar to spores of *Cladosporium*, and they identified these fungi as stages of the same mold. However, quoting from Bennett (**5**) "there is much evidence that Laurent's view is erroneous." Many plate cultures of *Pullularia* and of *Cladosporium* made in the course of the studies reported here clearly indicate, in agreement with Loew, Janczewski, Planchon, Hoggan, Brooks and Hansford and Bennett, as reported by Bennett (**5**), that there is no genetic relationship between the two, although frequently found growing together.

several days. Under favorable conditions this milky, mucoid growth can spread in four or five days to cover over a half or more of the agar surface in a 100 mm. petri dish culture. At this stage, but usually a little later, fine, radiating, colorless, submerged hyphae with attached conidia can be distinguished by a careful adjustment of lateral, reflected illumination against a black background under the higher powers of binocular magnification. This is especially well seen through the bottom of the petri dish. However, the great mass of conidia is produced by yeast-like budding of the conidia, which process, at a slightly later stage, gives rise to masses of these colorless conidia along the fine colorless hyphae, thus producing, near the center of the growth, broad, radiating bands of mucoid-milky growth tapering toward the distal ends of the hyphae and with frequent, tuberculoid enlargements where exceptionally rapid budding has taken place. This latter development takes place primarily if not entirely along the hyphae which radiate more deeply into the agar. In eight to ten days or earlier, a fringe of small, colorless hyphae usually more or less surrounds the mucoid to dark central area (FIG. 2, A), which may have also begun to pass into the next stage. At this time the whole culture may be entirely mucoid-milky in appearance, or fringed with hyphae, or there may be the beginning of the darkening associated with the production of brown and later black spores and hyphae (FIG. 2, B). This dark coloration may begin as a fine, scattered-dotted condition due to single scattered chlamydospores (FIG. 2, C). Further blackening is due either to dark walled hyphae (FIG. 2, A, D, E) changing rapidly to almost black and usually containing closely successive chlamydospores, or to the production of oval, brown spores similar in type to the colorless oval blastospores and forming broad bands of deep brown mucoid growth tapering toward the distal ends of the hyphae and with frequent tuberculoid enlargements (FIG. 2, D, E). In the still older cultures the entire growth may form a solid black mass including the submerged and surface layers (FIG. 2, F). The dark spores may vary from smooth to warty and may be single, in pairs, or in clumps of three to several (FIG. 1, G; FIG. 2, C, G). When formed within the hypha they may be single or from two to numerous and contiguous, or

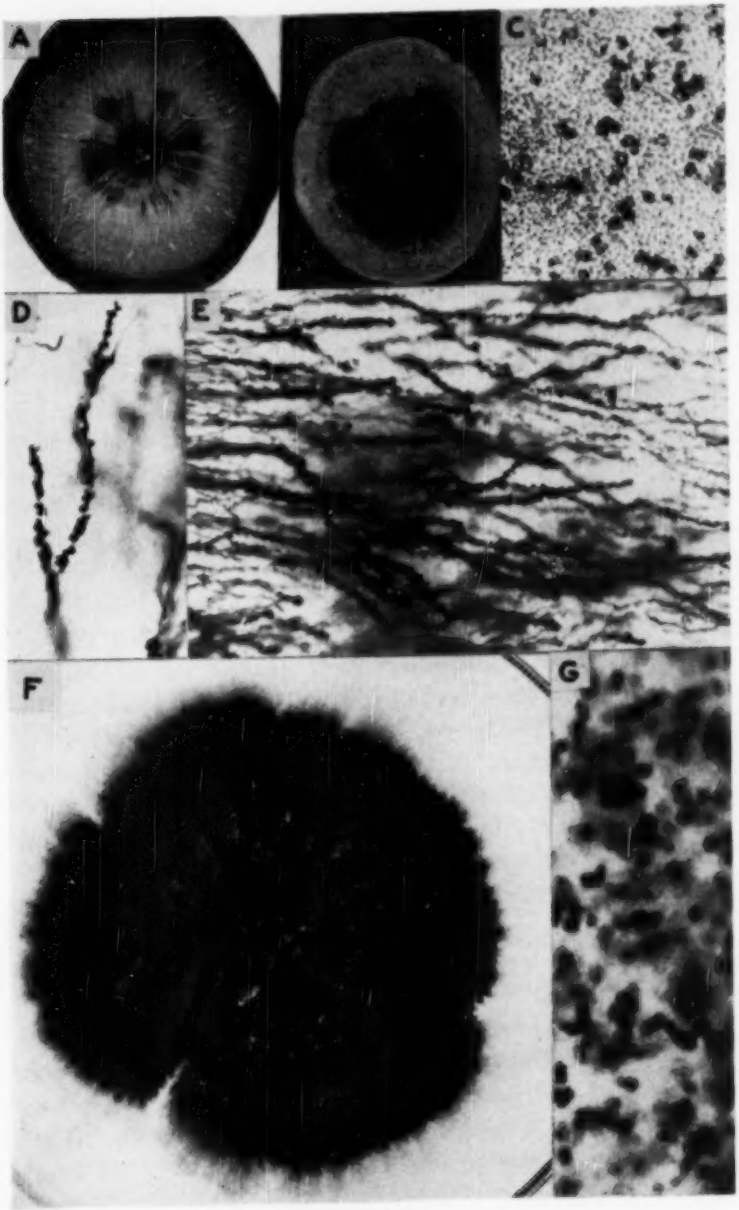

FIG. 2. *Pullularia* in culture.

sometimes with alternating groups of light colored vegetative cells of variable number between the groups of dark spores. It has been noted that a rapid blackening of the cultures occurs whenever somewhat unfavorable conditions exist, including competition with other molds on the same plate and drier conditions near the edge of the plate or where a thin agar layer is present. This reaction may be associated with the ability to resist unfavorable natural conditions such as those on the paint and plastic panels.

DISCUSSION

Resistant Nature of Pullularia. From a tabulation of reports on the occurrence of *Pullularia*, it appears that this fungus may be found especially abundantly in the sub-tropical, humid areas of the world but also frequently in the temperate, humid regions. However, its abundance on the upper, plastic surfaces, as viable inoculum, exposed facing the main source of heat and light in the Miami area, indicates that it is, under these conditions, highly resistant to high temperature, dryness and strong sunlight and can develop spores and hyphae (FIG. 1, G & H) during the brief periods of moisture, especially dew, characteristic of this area. On the other hand, Rennerfelt (**38**) reports that *Pullularia* grows best at about 27° C. on the synthetic medium which he used. It is not certain just how the dark, heavy-walled spores are produced on the paint and plastic surfaces. They may result from a very sparse mycelial development changing almost immediately into the heavy-walled chlamydospore stage, as seen in figure 1, H. Microscopic evidence from culture work indicates that this process can occur very early under unfavorable conditions and the fact that these specks on the paint and plastic surfaces are composed of small clumps and short chains of spores indicates that such mycelium as is formed rapidly attains the chlamydospore stage. Two physiological characteristics of *Pullularia* which may help to account for its ability to develop on paint and plastic surfaces outdoors are its dark colored spores, which are reported to be relatively more resistant to ultra-violet radiation than light colored spores (**13, 20, 44**), and its reported ability to assimilate elemental nitrogen (**43**). In addition to the early isolations of *Pullularia*

from the paint surfaces, later tests (August to November 1948) gave rise to colonies on agar plates in all cases, indicating long continued viability of the spores under these conditions.

Dissemination of Pullularia. Since the distribution of *Pullularia* infection spots on the paint and plastic surfaces is fairly uniform over the areas affected, it points to the probable spread of the spores by air currents rather than by animal agencies or rain. The fact that the heaviest infestation on the long paint panels was on the upper portions substantiates this view as does also the fact that the infestations were not in lines or in short trails such as one finds when fungi are distributed by crawling insects.

The source of the inoculum for these infestations is at present unknown since the paint panels and the plastic panels were both in open exposure sites, but at a distance of several miles from one another. In an informal report it has been stated to the author that in the course of allergenic studies being carried on in Miami, *Pullularia* has not been recognized as a common mold in the types caught on exposed agar plates. It has not been possible as yet for the writer to carry out tests of this type. The fact that neither of the paint tests showed any serious infestation until the second year of exposure indicates that there may have been very little *Pullularia* distribution during the first year (1946), and considerable during the second year of exposure (1947), or growth conditions were very different throughout these two years. The plastic samples were placed on test in January 1948 and had acquired a rather heavy infestation when the photograph (FIG. 1, C, D) was taken in April. Hence, the early part of that year was favorable for *Pullularia* distribution and development. In the Central and Southwestern United States, it is reported (**29**) that *Pullularia* may occur as a "shower" "and make up a significant fraction of the total mold count" of air-borne molds, although it is not listed by these authors as an allergenic mold. *Pullularia* has been reported as a soil inhabitant (**32, 42, 48**), but there seem to be no published data of its multiplication in the soil, or of any great abundance in that medium. In the case of the paint tests, at least, it appears improbable that the heavy infestation could have been from adjacent soil sources, since these tests were on exposure near the

shore of Biscayne Bay and not near any source of open soil. Moreover, there was very little evidence of soil particles on the paint samples and the heaviest infestation was at the upper ends of the panels. From these data it appears that although this mold is not uniformly an air constituent it may from time to time be carried in considerable amounts, possibly for considerable distances. Experience in Europe (**8, 40, 45**) has shown also that the presence of *Pullularia* as spores in the air and as sooty-mold is spasmodic or seasonal, but the sources of these spores do not appear to have been determined. The source and type of inoculum are still problematical where this mold develops in outdoor locations. It is possible, although by no means certain, that each small spot of infestation, on these paint and plastic panels, came from an original air-borne inoculum. However, it was clear from an examination of the plastic panels that some growth had taken place at many of the centers of infestation.

A variable ability of different cultures of *Pullularia* to produce the black pigment and numerous chlamydospores has been noted, but no specific studies on this have been made by the writer. It is stated by one investigator (**37**) that this organism may be able to synthesize to some extent the substances necessary for growth, but not for spores and color of mycelium.

SUMMARY AND CONCLUSIONS

The evidence presented in the current studies shows that this mold may be responsible for serious deterioration of painted surfaces in southern Florida quite aside from and prior to general climatic injury and is therefore an important subject of study for methods of prevention in order to prolong the early life of an otherwise satisfactory paint.

It is also demonstrated that certain plastic surfaces are likewise subject to serious deterioration by this same organism either associated with or prior to general climatic injury. The extent to which different types of plastics and plastic surfaces are subject to this type of deterioration should be given separate and careful consideration in the attempt to provide satisfactory plastic materials for use in the areas where this mold is prevalent.

The ready recognition of *Pullularia* as an organism causing injury or deterioration depends upon a knowledge of its characteristic appearance and stages under varying conditions, since, as indicated above, it is able to adapt itself readily to conditions which in general are unfavorable to most molds. Data and observations are included to provide some of this information. Further studies are in progress to round out the knowledge of its developmental stages under adverse conditions and the source and dissemination of the inoculum for the heavy infestations which occur in the open.

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EXPLANATION OF FIGURES

FIG. 1. Photomicrographs showing deterioration of paint and plastic surfaces due to *Pullularia* and other Dematiaceae. A. (1×) White painted panel showing heavy spotting with *Pullularia*. B. (1×) Panel painted with a different paint at the same time and exposed simultaneously with (A), but without *Pullularia* infestation. C. ($\frac{1}{5}$×) Plastic panel with a small amount of fungistat present. D. ($\frac{1}{5}$×) Untreated plastic panel, but both C and D heavily infested with *Pullularia* after three months' exposure. E. (8×) A portion of (A) showing various sizes of patches of the mold with the background of otherwise undeteriorated paint surface. F. (60×) A portion of (D) showing various sized patches of the mold. G (225×) Patches of *Pullularia* removed from a plastic panel by a collodion peel showing spore clumps of the "granular type" of growth. H. (225×) *Pullularia* in a collodion peel from a plastic panel showing detail of the "mycelial type" of growth. I. (60×) A portion of (D) showing patches of the mold variously connected by mycelial strands. J. (1×) A type of deterioration of another resin-impregnated glass cloth associated with an infestation of dematiaceous molds. K. (10×) Portion of (J) showing the elongated, black streaks of fungal growth following and intermixed with the glass fibers. L. (8×) A very young "mucoid" growth of *Pullularia* on potato dextrose agar with a small black center. Note the radiating hyphae with large masses of milky blastospores forming the blunt irregular marginal points with the hyphal tips projecting.

FIG. 2. Photomicrographs showing certain characteristics of *Pullularia* on potato dextrose agar medium. A. (2×) A young growth, older than in figure 1, L, with a white, radiating margin of hyphae and black mature center with radiating dark hyphae which finally become amalgamated into a solid black mature growth. B. (1×) A later stage showing the light young marginal area composed of hyphae and closely packed blastospores and the dark, mature central area. C. (225×) A very young growth removed and slightly flattened under a cover glass showing the masses of colorless blastospores and scattered dark, chlamydospores developed directly in the blastospore "mucoid" mass with no hyphal development. D. (30×) Dark hyphae with numerous scattered "tuberculoid" masses of dark blastospore-like spores as seen through the petri dish bottom against a light background largely composed of blastospores together with fine hyaline hyphae. E. (60×) A portion of (D). Colorless "tuberculoid" hyphae of otherwise similar type are developed in the younger stages of growth and may be found in the same petri dish culture with the dark ones shown here. F. (1×) A mature culture in which essentially all of the hyphae and spores are deep brown to black and with heavy walls. G. (225×) Short chains and clumps of dark chlamydospores with numerous colorless blastospores forming the background. A later stage than shown (FIG. 2, C).

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